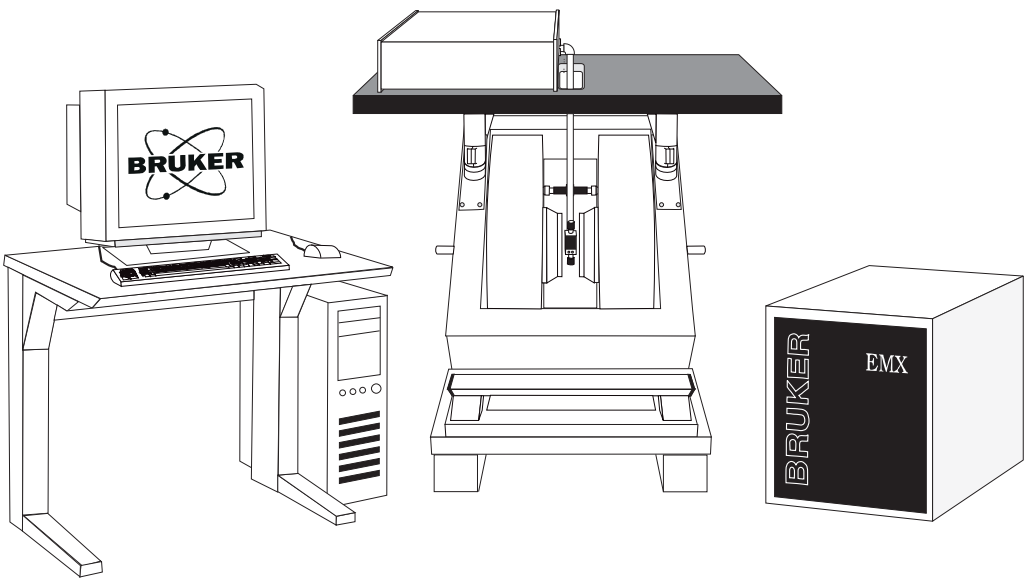
EMX USER'S MANUAL



EMX USER'S MANUAL

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EMX User's Manual
Manual Version 2.0
Software Version 2.3

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This manual is part of the original documentation for the Bruker EMX spectrometer.

Preface 0

Bruker strives to supply you with instructional and accurate documentation. We encourage you to tell us how we are doing. Please send us your suggestions for improvements, corrections, or bug reports. If there is anything you particularly liked, tell us as well. With your input and assistance, Bruker can continually improve its products and documentation.

You can send your messages and correspondence via e-mail, FAX, telephone, or mail. It is important to include the document name, product name, version number, and page number in your response. Here are the addresses and numbers to which you can send your messages.

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Thank you for your help.

Electrical Safety 0.1

Do not remove any of the protective covers or panels of the instrument. They are fitted to protect you and should be opened by qualified service personnel only.

Power off the instrument and disconnect the line cord before starting any cleaning work in the spectrometer. Never operate the instrument with the grounding cord disconnected or by passed. Facility wiring must include a properly grounded power receptacle.

Chemical Safety 0.2

Individuals working with hazardous chemicals, toxic substances, or enclosed liquid samples must take every precaution possible to avoid exposure to these agents. As a general rule, THINK OF THE CHEMICAL LABORATORY AS A HAZARDOUS ENVIRONMENT IN WHICH YOU MUST CONTINUALLY MAINTAIN A HIGH STANDARD OF VIGILANCE. Do not assume a cavalier attitude -- the substances with which you work present very real, and very serious threats to your health and safety.

Adhere to all currently recommended guidelines for standard laboratory safety as promulgated by governmental codes and contemporary laboratory practice. Inform yourself about the specific risks that are present when you handle actual or potential carcinogens (cancer-causing agents), explosive materials, strong acids, or any liquids that are sealed in glass containers.

Specifically:

- Be extremely careful when you handle sealed glass samples that are rapidly heated or cooled. The rapid cooling of some samples may result in the formation of a solid bolus in the sample tube that may make the tube prone to explosive rupture.

- Educate yourself about the temperature at which chemicals evaporate. When a sample gets close to the temperature at which it evaporates, it may quickly become volatile.

- In general, the safety threat posed by flying glass and violently escaping gases and liquids should not be underestimated.

- Wear safety glasses, face masks, and other protective clothing whenever there is any risk of spillage, breakage, or explosion. Protective shields should also be employed when there is any risk of explosion.

- Be sure that both storage and working areas are properly ventilated. They should be equipped with powerful blowers and fume heads.

- Store chemicals safely. Avoid integrating containers of chemicals that may result in dangerous combinations.

- Practice good housekeeping in work and storage areas. Clean up spills and refuse promptly. Do not leave volatile, combustible, or acidic liquids exposed on counters, benches, or other work areas.

- Make certain all chemical containers are properly labeled and classified, and that especially hazardous materials are appropriately designated with clearly understood decals or warnings.

- Never taste or inhale unmarked chemicals.
- All laboratories should be equipped with fire doors, fire extinguishers, fire smothering materials, and sprinkler systems or showers, as well as a detailed fire safety plan.

Microwave Safety 0.3

As long as the microwaves are contained in metal structures, microwaves can be very safe. Here are some precautions which, if followed, will eliminate the possibility of injury due to the microwaves.

- Do not have an open waveguide when the microwave power is on.
- Switch the bridge to standby when you remove or change EPR cavities.
- Never look down an open waveguide when there is microwave power. The eyes are very susceptible to damage from microwaves.

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Introduction 1

This introduction describes the operation of a Bruker EMX EPR (Electron Paramagnetic Resonance) spectrometer. No assumptions have been made about the background of the reader except that he or she has a general scientific or technical background. Many of the elementary principles necessary for following the chapters are presented in a concise form.

This chapter starts with a list of EPR applications. A brief description of the spectrometer and its capabilities follows. The chapter concludes with an explanation of how to use this manual.

EPR Applications 1.1

EPR has matured into a powerful, versatile, nondestructive, and nonintrusive analytical method. Unlike many other techniques, EPR yields meaningful structural and dynamical information, even from ongoing chemical or physical processes without influencing the process itself. Therefore, it is an ideal complementary technique for other methods in a wide range of studies and application areas. Here is a list of some of the EPR applications which are commonly used.

Chemistry 1.1.1

- Kinetics of radical reactions
- Polymerization reactions
- Spin trapping
- Organo-metallic compounds
- Catalysis
- Petroleum research
- Oxidation and reduction processes
- Biradicals and triplet states of molecules

Physics 1.1.2

- Measurement of magnetic susceptibility
- Transition metal, lanthanide, and actinide ions
- Conduction electrons in conductors and semiconductors
- Defects in crystals (e.g. color centers in alkali-halides)
- Optical detection of magnetic resonance, excited states of molecules
- Crystal fields in single crystals
- Recombination at low temperatures

Materials Research 1.1.3

- Degradation of paints and polymers by light
- Polymer properties
- Defects in diamond
- Defects in optical fibers
- Laser materials
- Organic conductors
- Influence of impurities and defects in semiconductors
- Properties of novel magnetic materials
- High T_C superconductors
- C_{60} compounds
- Behavior of free radicals in corrosion

Ionizing Radiation 1.1.4

- Alanine radiation dosimetry
- Control of irradiated foods
- Archaeological dating
- Short-time behavior of organic free radicals produced by radiation
- Radiation effects and damage
- Radiation effects on biological compounds

Biology and Medicine 1.1.5

- Spin label and spin probe techniques
- Spin trapping
- Dynamics of biomolecules using saturation transfer techniques
- Free radicals in living tissues and fluids
- Antioxidants, radical scavengers
- Contrast agents
- Oximetry
- Drug detection, metabolism, and toxicity
- Enzyme reactions
- Photosynthesis
- Structure and identification of metal-binding sites
- Photochemical and radiolytic generation of radicals
- Oxygen based radicals
- NO in biological systems
- Carcinogenic reactions

The Spectrometer 1.2

The Bruker EMX EPR spectrometer is a research grade scientific instrument. It is capable of routine measurements, as well as sophisticated and advanced experiments when equipped with the proper accessories. The modular design makes the spectrometer easy to upgrade or expand. For information and assistance in choosing and ordering accessories for your specific application, contact your local Bruker EPR sales representative.

Using this Manual 1.3

How to Find Things 1.3.1

Preface First, you should read the safety guide in the preface of the manual. Microwaves can be dangerous, particularly to your eyes. With normal precautions, the risk for injury can be minimized.

Chapter 2 Users who are not familiar with EPR should start by reading Chapter 2, which is a concise introduction to the theory and practice of EPR spectroscopy. It is by no means exhaustive; it gives the necessary information to follow the other chapters of the manual. A short list of references is given at the end of the chapter for more information.

Chapter 3 This chapter is a simple “how to” section describing how to acquire the spectrum of sample. It covers turning the spectrometer on, tuning the microwave cavity and bridge, and acquiring spectra. The step by step instructions lead you through the acquisition of a strong pitch (a standard sample) EPR signal.

Chapter 4 This tutorial introduces you to many of the convenient and commonly used features of the spectrometer.

Chapter 5 The most basic operations of the Bruker EPR spectrometer are covered in Chapter 3. Chapter 5 explains further procedures such as how to change cavities, how to tune a bridge manually, and how to perform 2-D experiments.

Chapter 6 General helpful hints for acquiring EPR spectra are presented in this chapter. Before consulting this chapter, you should be familiar with the material in Chapters 2 and 3 It gives tips on where to find EPR signals as well as how to optimize the sensitivity of the spectrometer for your particular sample.

Chapter 7 Sometimes, things go wrong. Chapter 7 gives some possible solutions to problems you may be having. Many times, problems appear to be the fault of the instrument; however, with the proper choice of operating conditions, these problems often disappear.

Chapter 8 This chapter describes the procedure for calibrating cavities and signal channels so that you may obtain reproducible, quantitative, and high sensitivity spectra.

Chapter 9 In order to maintain your spectrometer in optimal working condition, it is a good idea to periodically test your system's performance. Chapter 9 describes how to measure the sensitivity of the spectrometer and how to measure the background signal of the cavity.

Chapter 10 An extensive bibliography of EPR references is given in this chapter. It includes many different EPR applications as well as educational texts. This is a good place to start a literature search.

All the answers to all EPR questions can not possibly fit in one manual. If you can not find the answer to your question, contact your nearest Bruker EPR representative. We have a team of skilled application scientists with diverse expertises. One of us will probably come up with an answer.

Typographical Conventions 1.3.2

Special fonts are used in the text to differentiate between normal manual text and text displayed in the program.

Times This is the font used for the normal text in the manual.

Helvetica This is the font used for text that is displayed by the program or must be entered into the program by you.

An EPR Primer 2

This chapter is an introduction to the basic theory and practice of EPR spectroscopy. It gives you sufficient background to understand the following chapters. In addition, we strongly encourage the new user to explore some of the texts and articles at the end of this chapter. You can then fully benefit from your particular EPR application or think of new ones.

Basic EPR Theory 2.1

Introduction to Spectroscopy 2.1.1

During the early part of this century, when scientists began to apply the principles of quantum mechanics to describe atoms or molecules, they found that a molecule or atom has discrete (or separate) states, each with a corresponding energy. Spectroscopy is the measurement and interpretation of the energy differences between the atomic or molecular states. With knowledge of these energy differences, you gain insight into the identity, structure, and dynamics of the sample under study.

We can measure these energy differences, ΔE, because of an important relationship between ΔE and the absorption of electromagnetic radiation. According to Planck's law, electromagnetic radiation will be absorbed if:

$$\Delta E = h\nu \, , \qquad [2\text{-}1]$$

where h is Planck's constant and ν is the frequency of the radiation.

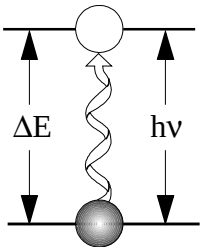


Figure 2-1 Transition associated with the absorption of electromagnetic energy.

The absorption of energy causes a transition from the lower energy state to the higher energy state. (See Figure 2-1.) In conventional spectroscopy, ν is varied or swept and the frequencies at which absorption occurs correspond to the energy differences of the states. (We shall see later that EPR differs slightly.) This record is called a spectrum. (See Figure 2-2.) Typically, the frequencies vary from the megahertz range for NMR (Nuclear Magnetic Resonance) (AM, FM, and TV transmissions use electromagnetic radiation at these frequencies), through visible light, to ultraviolet light. Radiation in the gigahertz range (the same as in your microwave oven) is used for EPR experiments.

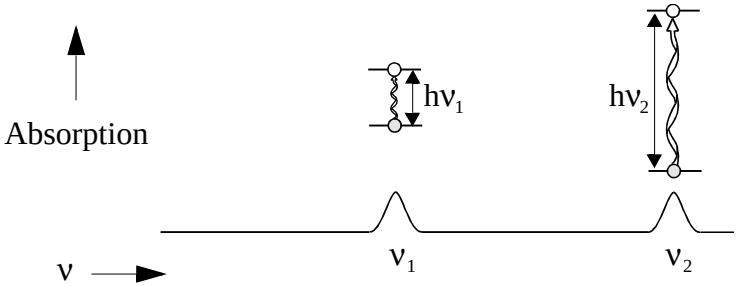


Figure 2-2 A spectrum.

The Zeeman Effect 2.1.2

The energy differences we study in EPR spectroscopy are predominately due to the interaction of unpaired electrons in the sample with a magnetic field produced by a magnet in the laboratory. This effect is called the Zeeman effect. Because the electron has a magnetic moment, it acts like a compass or a bar magnet when you place it in a magnetic field, B_0. It will have a state of lowest energy when the moment of the electron, μ, is aligned with the magnetic field and a state of highest energy when μ is aligned against the magnetic field. (See Figure 2-3.) The two states are labelled by the projection of the electron spin, M_s, on the direction of the magnetic field. Because the electron is a spin 1/2 particle, the parallel state is designated as $M_s = - 1/2$ and the antiparallel state is $M_s = + 1/2$.

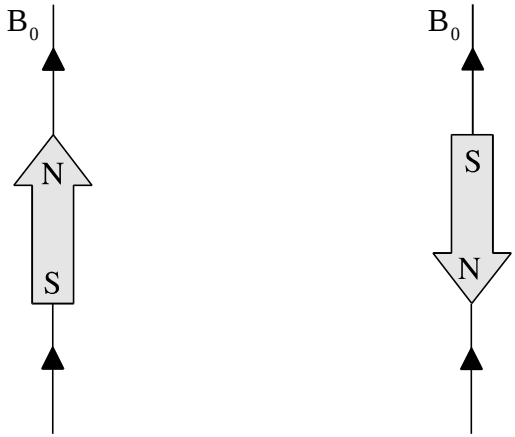


Figure 2-3 Minimum and maximum energy orientations of μ with respect to the magnetic field B_0.

From quantum mechanics, we obtain the most basic equations of EPR:

$$E = g\,\mu_B\,B_0\,M_s = \pm\frac{1}{2}\,g\,\mu_B\,B_0 \qquad [2\text{-}2]$$

and

$$\Delta E = h\nu = g\,\mu_B B_0. \qquad [2\text{-}3]$$

g is the g-factor, which is a proportionality constant approximately equal to 2 for most samples, but varies depending on the electronic configuration of the radical or ion. μ_B is the Bohr magneton, which is the natural unit of electronic magnetic moment.

Two facts are apparent from equations Equation [2-2] and Equation [2-3] and its graph in Equation Figure 2-4.

- The two spin states have the same energy in the absence of a magnetic field.
- The energies of the spin states diverge linearly as the magnetic field increases.

These two facts have important consequences for spectroscopy.

- Without a magnetic field, there is no energy difference to measure.
- The measured energy difference depends linearly on the magnetic field.

Because we can change the energy differences between the two spin states by varying the magnetic field strength, we have an alternative means to obtain spectra. We could apply a constant magnetic field and scan the frequency of the electromagnetic radiation as in conventional spectroscopy. Alternatively, we could keep the electromagnetic radiation frequency constant and scan the magnetic field. (See Figure 2-4.) A peak in the absorption will occur when the magnetic field “tunes” the two spin states so that their energy difference matches the energy of the radiation. This field is called the “field for resonance”. Owing to the limitations of microwave electronics, the latter method offers superior performance. This technique is used in all Bruker EPR spectrometers.

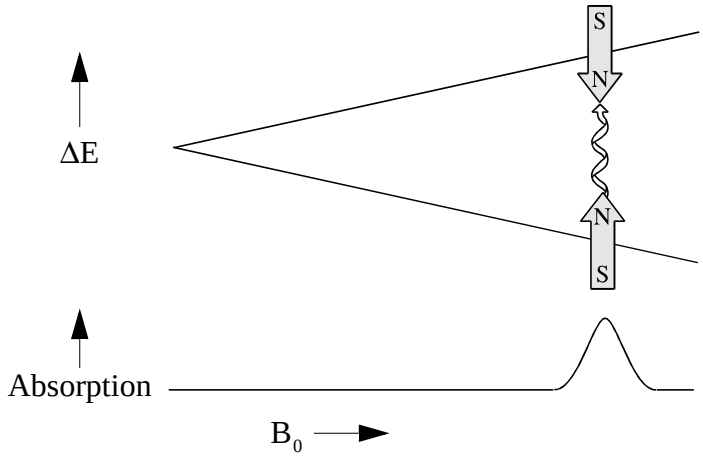


Figure 2-4 Variation of the spin state energies as a function of the applied magnetic field.

The field for resonance is not a unique “fingerprint” for identification of a compound because spectra can be acquired at several different frequencies. The g-factor,

$$g = \frac{h\nu}{\mu_B B_0} \quad , \qquad [2\text{-}4]$$

being independent of the microwave frequency, is much better for that purpose. Notice that high values of g occur at low magnetic fields and vice versa. A list of fields for resonance for a g = 2 signal at microwave frequencies commonly available in EPR spectrometers is presented in Table 2-1.

Microwave Band	Frequency (GHz)	B_{res} (G)
L	1.1	392
S	3.0	1070
X	9.75	3480
Q	34.0	12000
W	94.0	34000

Table 2-1 Field for resonance, B_{res}, for a g = 2 signal at selected microwave frequencies.

Hyperfine Interactions 2.1.3

Measurement of g-factors can give us some useful information; however, it does not tell us much about the molecular structure of our sample. Fortunately, the unpaired electron, which gives us the EPR spectrum, is very sensitive to its local surroundings. The nuclei of the atoms in a molecule or complex often have a magnetic moment, which produces a local magnetic field at the electron. The interaction between the electron and the nuclei is called the hyperfine interaction. It gives us a wealth of information about our sample such as the identity and number of atoms which make up a molecule or complex as well as their distances from the unpaired electron.

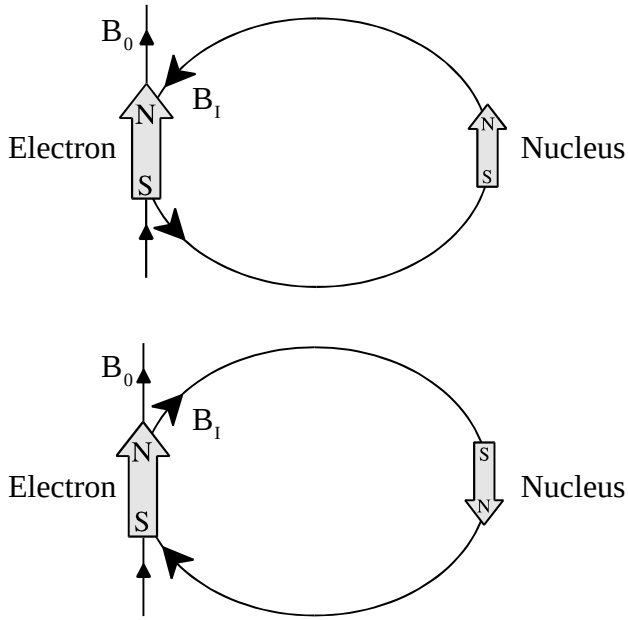


Figure 2-5 Local magnetic field at the electron, B_I, due to a nearby nucleus.

Equation Figure 2-5 depicts the origin of the hyperfine interaction. The magnetic moment of the nucleus acts like a bar magnet (albeit a weaker magnet than the electron) and produces a magnetic field at the electron, B_I. This magnetic field opposes or adds to the magnetic field from the laboratory magnet, depending on the alignment of the moment of the nucleus. When B_I adds to the magnetic field, we need less magnetic field from our laboratory magnet and therefore the field for resonance is lowered by B_I. The opposite is true when B_I opposes the laboratory field.

For a spin 1/2 nucleus such as a hydrogen nucleus, we observe that our single EPR absorption signal splits into two signals which are each B_I away from the original signal. (See Figure 2-6.)

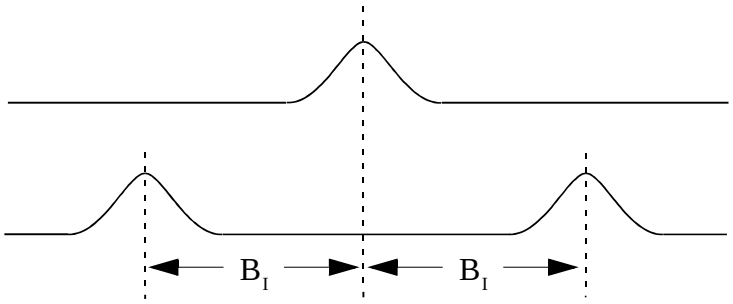


Figure 2-6 Splitting in an EPR signal due to the local magnetic field of a nearby nucleus.

If there is a second nucleus, each of the signals is further split into a pair, resulting in four signals. For N spin 1/2 nuclei, we will generally observe 2^N EPR signals. As the number of nuclei gets larger, the number of signals increases exponentially. Sometimes there are so many signals that they overlap and we only observe one broad signal.

Signal Intensity 2.1.4

So far, we have concerned ourselves with where the EPR signal is, but the size of the EPR signal is also important if we want to measure the concentration of the EPR active species in our sample. In the language of spectroscopy, the size of a signal is defined as the integrated intensity, i.e., the area beneath the absorption curve. (See Figure 2-7.) The integrated intensity of an EPR signal is proportional to the concentration.

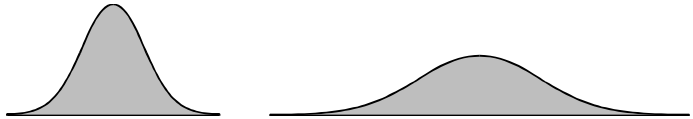


Figure 2-7 Integrated intensity of absorption signals. Both signals have the same intensity.

Signal intensities do not depend solely on concentrations. They also depend on the microwave power. If you do not use too much microwave power, the signal intensity grows as the square root of the power. At higher power levels, the signal diminishes as well as broadens with increasing microwave power levels. This effect is called saturation. If you want to measure accurate linewidths, lineshapes, and closely spaced hyperfine splittings, you should avoid saturation by using low microwave power. A quick means of checking for the absence of saturation is to decrease the microwave power and verify that the signal intensity also decreases by the square root of the microwave power.

Basic EPR Practice 2.2

Introduction to Spectrometers 2.2.1

In the first half of this chapter, we discussed the theory of EPR spectroscopy. Now we need to consider the practical aspects of EPR spectroscopy. Theory and practice have always been strongly interdependent in the development and growth of EPR. A good example of this point is the first detection of an EPR signal by Zavoisky in 1945. The Zeeman effect had been known in optical spectroscopy for many years, but the first direct detection of EPR had to wait until the development of radar during World War II. Only then, did scientists have the necessary components to build sufficiently sensitive spectrometers (scientific instruments designed to acquire spectra). The same is true today with the development of advanced techniques in EPR such as Fourier Transform and high frequency EPR.

The simplest possible spectrometer has three essential components: a source of electromagnetic radiation, a sample, and a detector. (See Figure 2-8.) To acquire a spectrum, we change the frequency of the electromagnetic radiation and measure the amount of radiation which passes through the sample with a detector to observe the spectroscopic absorptions. Despite the apparent complexities of any spectrometer you may encounter, it can always be simplified to the block diagram shown in Figure 2-8.

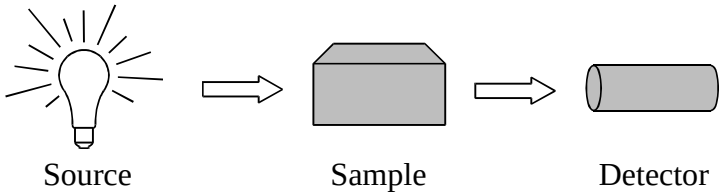


Figure 2-8 The simplest spectrometer.

Figure 2-9 shows the general layout of a Bruker EPR spectrometer. The electromagnetic radiation source and the detector are in a box called the “microwave bridge”. The sample is in a microwave cavity, which is a metal box that helps to amplify weak signals from the sample. As mentioned in Section 2.1.2, there is a magnet to “tune” the electronic energy levels. In addition, we have a console, which contains signal processing and control electronics and a computer. The computer is used for analyzing data as well as coordinating all the units for acquiring a spectrum. In the following sections you will become acquainted with how these different parts of the spectrometer function and interact.

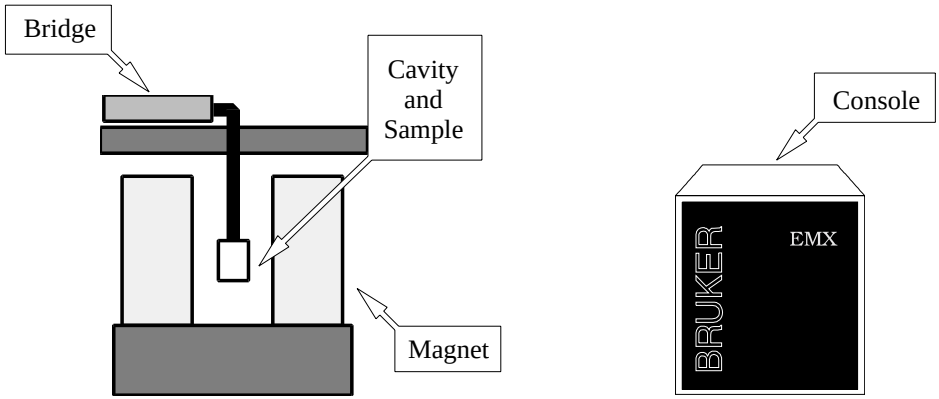


Figure 2-9 The general outlay of an EPR spectrometer.

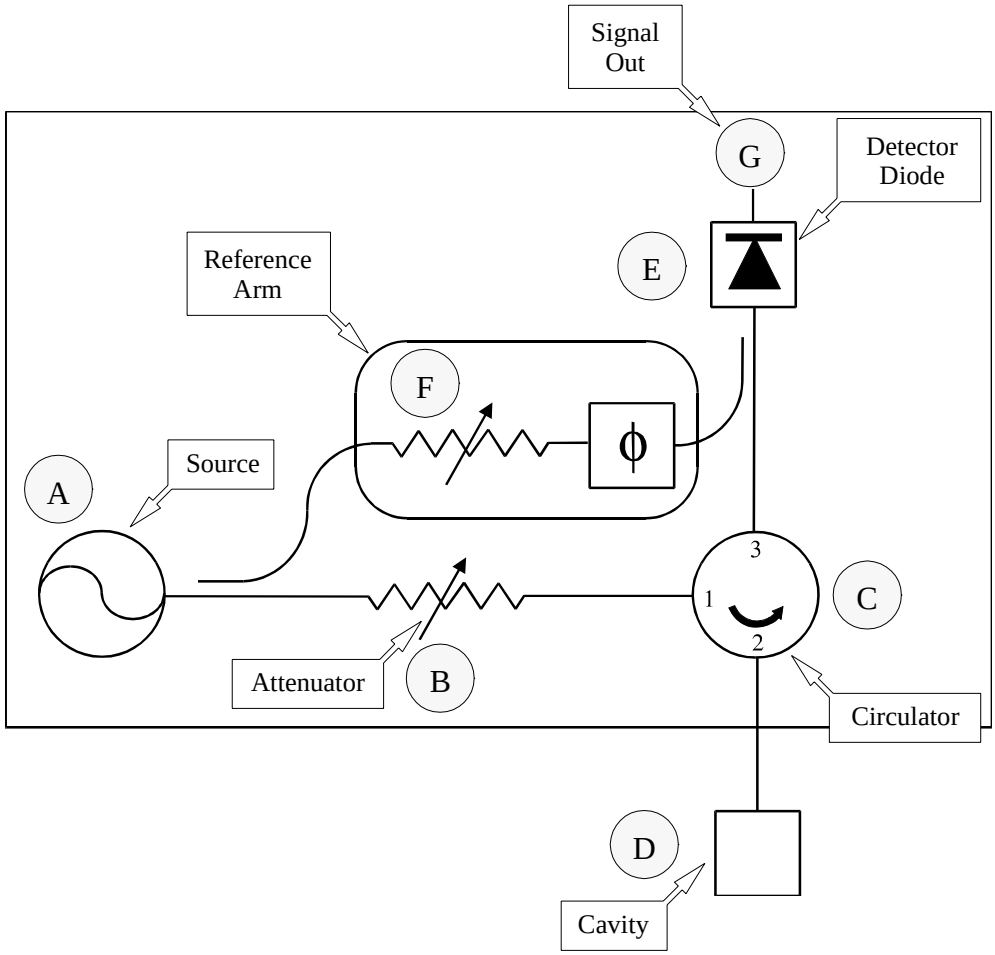


Figure 2-10 Block diagram of a microwave bridge.

The Microwave Bridge 2.2.2

The microwave bridge houses the microwave source and the detector. There are more parts in a bridge than shown in Figure 2-10, but most of them are control, power supply, and security electronics and are not necessary for understanding the basic operation of the bridge. We shall now follow the path of the microwaves from the source to the detector.

We start our tour of the microwave bridge at point A, the microwave source. The output power of the microwave source cannot be varied easily, however in our discussion of signal intensity, we stressed the importance of changing the power level. Therefore, the next component, at point B, after the microwave source is a variable attenuator, a device which blocks the flow of microwave radiation. With the attenuator, we can precisely and accurately control the microwave power which the sample sees.

Bruker EPR spectrometers operate slightly differently than the simple spectrometer shown in the block diagram, Figure 2-8. The diagram depicts a transmission spectrometer (It measures the amount of radiation transmitted through the sample.) and most EPR spectrometers are reflection spectrometers. They measure the changes (due to spectroscopic transitions) in the amount of radiation reflected back from the microwave cavity containing the sample (point D in the figure). We therefore want our detector to see only the microwave radiation coming back from the cavity. The circulator at point C is a microwave device which allows us to do this. Microwaves coming in port 1 of the circulator only go to the cavity through port 2 and not directly to the detector through port 3. Reflected microwaves are directed only to the detector and not back to the microwave source.

We use a Schottky barrier diode to detect the reflected microwaves (point E in the figure). It converts the microwave power to an electrical current. At low power levels, (less than 1 microwatt) the diode current is proportional to the microwave power and the detector is called a square law detector. (Remember that

electrical power is proportional to the square of the voltage or current.) At higher power levels, (greater than 1 milliwatt) the diode current is proportional to the square root of the microwave power and the detector is called a linear detector. The transition between the two regions is very gradual.

For quantitative signal intensity measurements as well as optimal sensitivity, the diode should operate in the linear region. The best results are attained with a detector current of approximately 200 microamperes. To insure that the detector operates at that level, there is a reference arm (point F in the figure) which supplies the detector with some extra microwave power or “bias”. Some of the source power is tapped off into the reference arm, where a second attenuator controls the power level (and consequently the diode current) for optimal performance. There is also a phase shifter to insure that the reference arm microwaves are in phase with the reflected signal microwaves when the two signals combine at the detector diode.

The detector diodes are very sensitive to damage from excessive microwave power and will slowly lose their sensitivity. To prevent this from happening, there is protection circuitry in the bridge which monitors the current from the diode. When the current exceeds 400 microamperes, the bridge automatically protects the diode by lowering the microwave power level. This reduces the risk of damage due to accidents or improper operating procedures. However, it is good lab practice to follow correct procedures and not rely on the protection circuitry.

The EPR Cavity 2.2.3

In this section, we shall discuss the properties of microwave (EPR) cavities and how changes in these properties due to absorption result in an EPR signal. We use microwave cavities to amplify weak signals from the sample. A microwave cavity is simply a metal box with a rectangular or cylindrical shape which resonates with microwaves much as an organ pipe resonates with sound waves. Resonance means that the cavity stores the microwave energy; therefore, at the resonance frequency of the cavity, no microwaves will be reflected back, but will remain inside the cavity. (See Figure 2-11.)

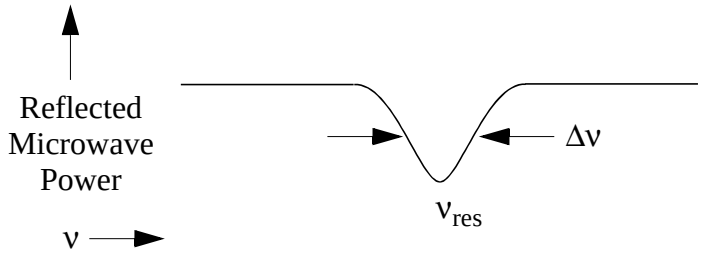


Figure 2-11 Reflected microwave power from a resonant cavity.

Cavities are characterized by their Q or quality factor, which indicates how efficiently the cavity stores microwave energy. As Q increases, the sensitivity of the spectrometer increases. The Q factor is defined as

$$Q = \frac{2\pi \text{ (energy stored)}}{\text{energy dissipated per cycle}} , \qquad [2\text{-}5]$$

where the energy dissipated per cycle is the amount of energy lost during one microwave period. Energy can be lost to the side walls of the cavity because the microwaves generate electrical currents in the side walls of the cavity which in turn generates

heat. We can measure Q factors easily because there is another way of expressing Q:

$$Q = \frac{\nu_{res}}{\Delta\nu} \quad , \qquad [2\text{-}6]$$

where ν_{res} is the resonant frequency of the cavity and $\Delta\nu$ is the width at half height of the resonance.

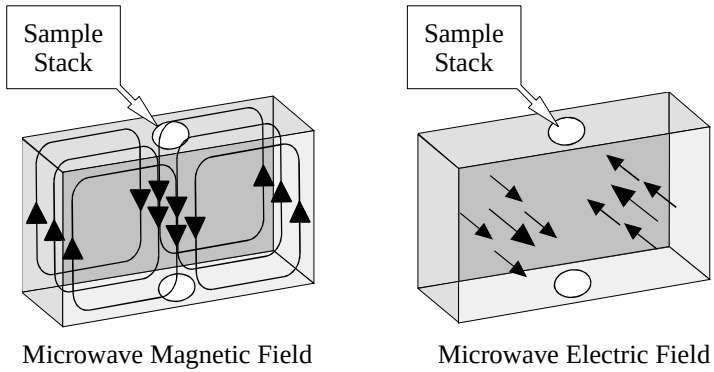


Figure 2-12 Magnetic and electric field patterns in a standard EPR cavity.

A consequence of resonance is that there will be a standing wave inside the cavity. Standing electromagnetic waves have their electric and magnetic field components exactly out of phase, i.e. where the magnetic field is maximum, the electric field is minimum and vice versa. The spatial distribution of the amplitudes of the electric and magnetic fields in the most commonly used EPR cavity is shown in Figure 2-12. We can use the spatial separation of the electric and magnetic fields in a cavity to great advantage. Most samples have non-resonant absorption of the microwaves via the electric field (this is how a microwave oven works) and the Q will be degraded by an increase in the dissipated energy. It is the magnetic field that drives the absorption in

EPR. Therefore, if we place our sample in the electric field minimum and the magnetic field maximum, we obtain the biggest signals and the highest sensitivity. The cavities are designed for optimal placement of the sample.

We couple the microwaves into the cavity via a hole called an iris. The size of the iris controls the amount of microwaves which will be reflected back from the cavity and how much will enter the cavity. The iris accomplishes this by carefully matching or transforming the impedances (the resistance to the waves) of the cavity and the waveguide (a rectangular pipe used to carry microwaves). There is an iris screw in front of the iris which allows us to adjust the “matching”. This adjustment can be visualized by noting that as the screw moves up and down, it effectively changes the size of the iris. (See Figure 2-13.)

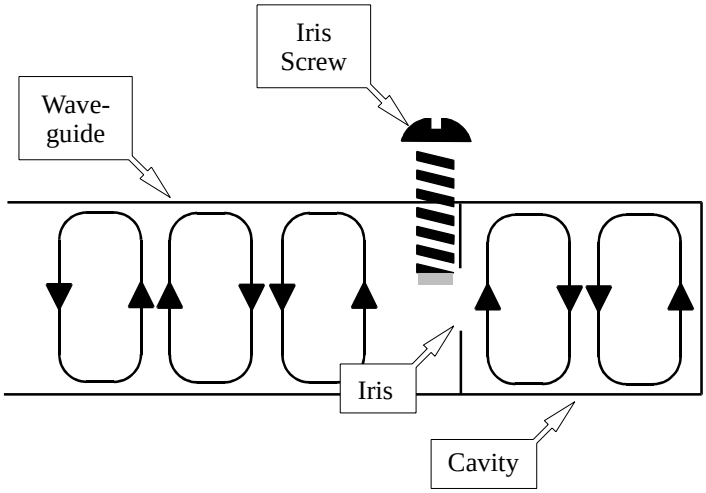


Figure 2-13 The matching of a microwave cavity to waveguide.

How do all of these properties of a cavity give rise to an EPR signal? When the sample absorbs the microwave energy, the Q is lowered because of the increased losses and the coupling

changes because the absorbing sample changes the impedance of the cavity. The cavity is therefore no longer critically coupled and microwave will be reflected back to the bridge, resulting in an EPR signal.

The Signal Channel 2.2.4

EPR spectroscopists use a technique known as phase sensitive detection to enhance the sensitivity of the spectrometer. The advantages include less noise from the detection diode and the elimination of baseline instabilities due to the drift in DC electronics. A further advantage is that it encodes the EPR signals to make it distinguishable from sources of noise or interference which are almost always present in a laboratory. The signal channel, a unit which fits in the spectrometer console, contains the required electronics for the phase sensitive detection.

The detection scheme works as follows. The magnetic field strength which the sample sees is modulated (varied) sinusoidally at the modulation frequency. If there is an EPR signal, the field modulation quickly sweeps through part of the signal and the microwaves reflected from the cavity are amplitude modulated at the same frequency. For an EPR signal which is approximately linear over an interval as wide as the modulation amplitude, the EPR signal is transformed into a sine wave with an amplitude proportional to the slope of the signal (See Figure 2-14.)

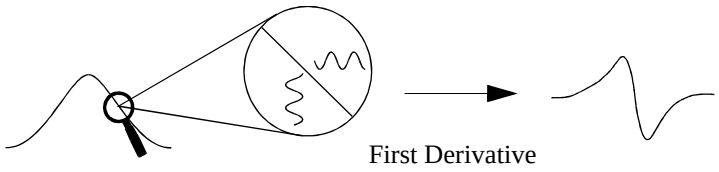


Figure 2-14 Field modulation and phase sensitive detection.

The signal channel (more commonly known as a lock-in amplifier or phase sensitive detector) produces a DC signal proportional to the amplitude of the modulated EPR signal. It compares the modulated signal with a reference signal having the same frequency as the field modulation and it is only sensitive to signals which have the same frequency and phase as the field modulation. Any signals which do not fulfill these requirements (i.e., noise and electrical interference) are suppressed. To further improve the sensitivity, a time constant is used to filter out more of the noise.

Phase sensitive detection with magnetic field modulation can increase our sensitivity by several orders of magnitude; however, we must be careful in choosing the appropriate modulation amplitude, frequency, and time constant. All three variables can distort our EPR signals and make interpretation of our results difficult.

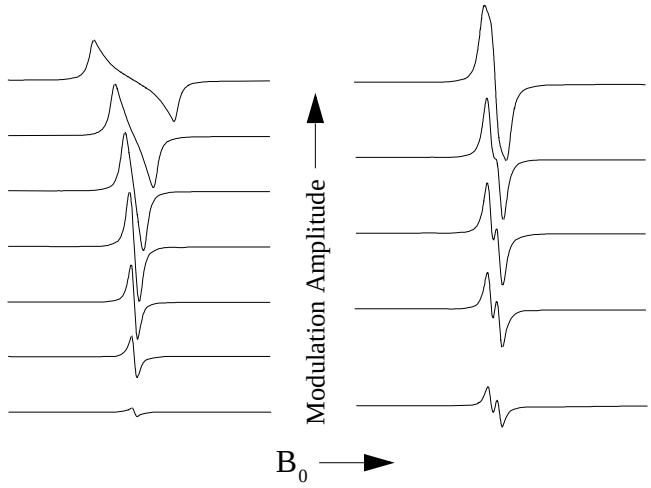


Figure 2-15 Signal distortions due to excessive field modulation.

As we apply more magnetic field modulation, the intensity of the detected EPR signals increases; however, if the modulation amplitude is too large (larger than the linewidths of the EPR signal), the detected EPR signal broadens and becomes distorted. (See Figure 2-15.) A good compromise between signal intensity and signal distortion occurs when the amplitude of the magnetic field modulation is equal to the width of the EPR signal. Also, if we use a modulation amplitude greater than the splitting between two EPR signals, we can no longer resolve the two signals.

Time constants filter out noise by slowing down the response time of the spectrometer. As the time constant is increased, the noise levels will drop. If we choose a time constant which is too long for the rate at which we scan the magnetic field, we can distort or even filter out the very signal which we are trying to extract from the noise. Also, the apparent field for resonance will shift. Figure 2-16 shows the distortion and disappearance of a signal as the time constant is increased. If you need to use a long time constant to see a weak signal, you must use a slower scan rate. A safe rule of thumb is to make sure that the time needed to scan through a single EPR signal should be ten times greater than the length of the time constant.

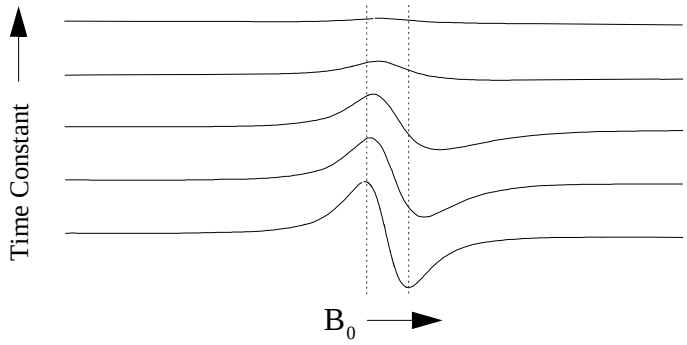


Figure 2-16 Signal distortion and shift due to excessive time constants.

For samples with very narrow or closely spaced EPR signals, (~ 50 milligauss. This usually only happens for organic radicals in dilute solutions.) we can get a broadening of the signals if our modulation frequency is too high (See Figure 2-17.) The broadening is a consequence of the Heisenberg uncertainty principle.

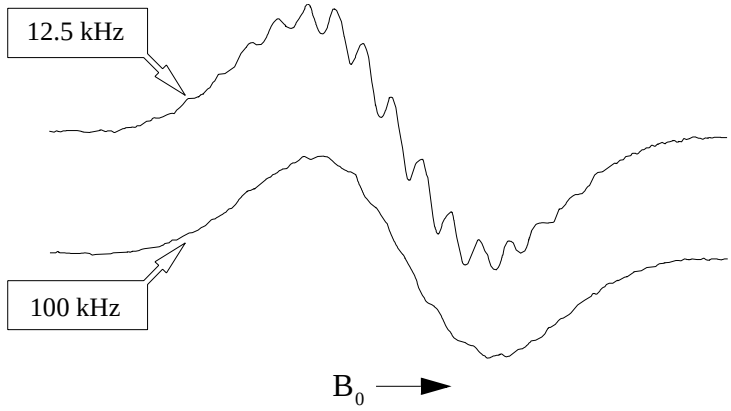


Figure 2-17 Loss of resolution due to high modulation frequency.

The Magnetic Field Controller 2.2.5

The magnetic field controller allows us to sweep the magnetic field in a controlled and precise manner for our EPR experiment. It consists of two parts; a part which sets the field values and the timing of the field sweep and a part which regulates the current in the windings of the magnet to attain the requested magnetic field value.

The magnetic field values and the timing of the magnetic field sweep are controlled by a microprocessor in the controller. A field sweep is divided into a maximum of 4096 discrete steps called sweep addresses. At each step, a reference voltage corresponding to the magnetic field value is sent to the part of the controller that regulates the magnetic field. The sweep rate is

controlled by varying the waiting time between the individual steps.

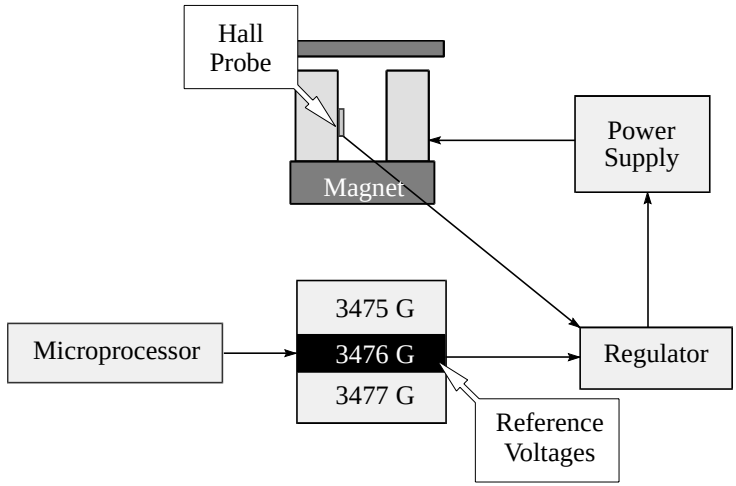


Figure 2-18 A block diagram of the field controller and associated components.

The magnetic field regulation occurs via a Hall probe placed in the gap of the magnet. It produces a voltage which is dependent on the magnetic field perpendicular to the probe. The relationship is not linear and the voltage changes with temperature; however, this is easily compensated for by keeping the probe at a constant temperature slightly above room temperature and characterizing the nonlinearities so that the microprocessor in the controller can make the appropriate corrections. Regulation is accomplished by comparing the voltage from the Hall probe with the reference voltage given by the other part of the controller. When there is a difference between the two voltages, a correction voltage is sent to the magnet power supply which changes the amount of current flowing through the magnet windings and hence the magnetic field. Eventually the error

voltage drops to zero and the field is “stable” or “locked”. This occurs at each discrete step of a magnetic field scan.

The Spectrum 2.2.6

We have seen how the individual components of the spectrometer work. Figure 2-19 shows how they work together to produce a spectrum.

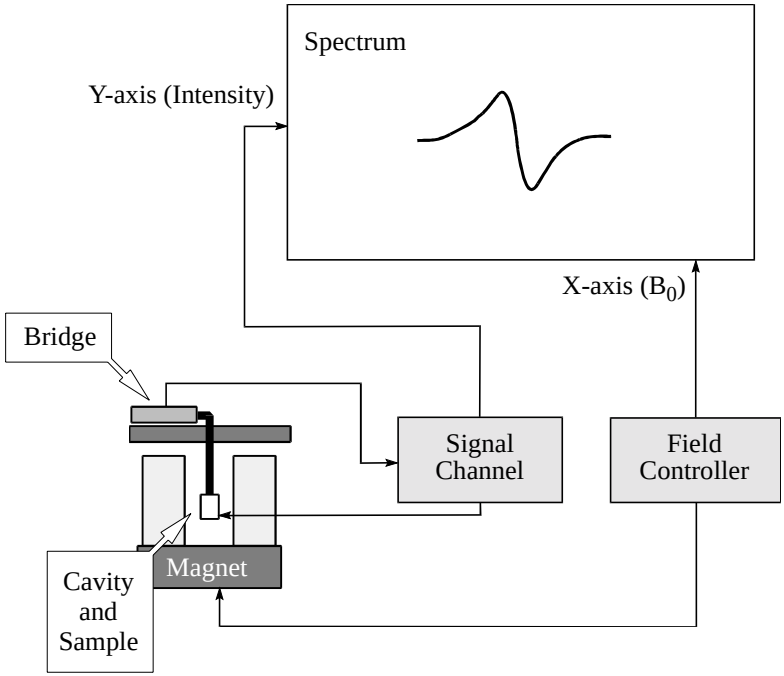


Figure 2-19 Block diagram of an EPR spectrometer.

Suggested Reading 2.3

This chapter is a brief overview of the basic theory and practice of EPR spectroscopy. If you would like to learn more, there are many good books and articles that have been written on these subjects. We recommend the following:

Instrumentation:

Poole, C. *Electron Spin Resonance a Comprehensive Treatise on Experimental Techniques, Editions 1,2:* Interscience Publishers, New York, (1967), (1983).

Feher, G. *Sensitivity Considerations in Microwave Paramagnetic Resonance Absorption Techniques:* Bell System Tech. J. 36, 449 (1957).

Theory:

Knowles, P.F., D. Marsh and H.W.E. Rattle. *Magnetic Resonance of Biomolecules:* J. Wiley, New York, (1976).

Weil, John A., J.R. Bolton, and Wertz, J.E., *Electron Paramagnetic Resonance, Elementary Theory and Practical Applications:* Wiley-Interscience, New York, (1994).

A more extensive bibliography is found in last chapter of this manual.

Getting Started 3

This chapter contains basic operating instructions for first time users of a Bruker EMX spectrometer. It describes basic operation of an EMX spectrometer with an X-band bridge (9.1-9.9 GHz). This chapter will guide you from a completely shut down spectrometer to a hardcopy of your spectrum on a printer. You will learn to acquire an EPR spectrum of a standard sample with the WIN-EPR Acquisition Software. There will also be recommended precautions to prevent damage to the instrument. All the components possess self-protecting features; however, it is good lab practice to follow correct operating procedures and not rely on the protection circuitry. No in-depth knowledge of EPR is required; however we recommend that you familiarize yourself with some of the material in Chapter 2. To help you in the following sections, Figure 3-1 will assist you in identifying the various units which comprise the EPR spectrometer.

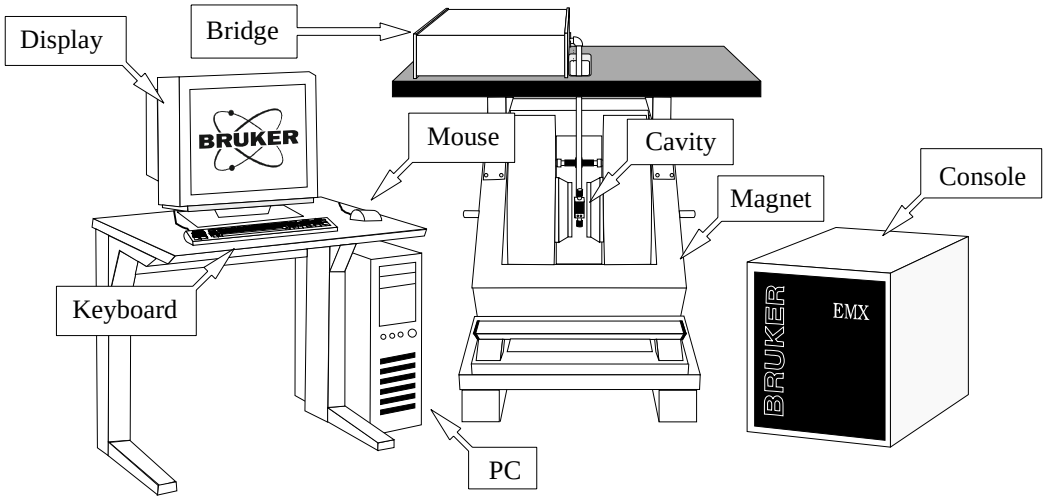


Figure 3-1 Modules and components of the EMX spectrometer.

Brief Tips on Windows™ 95 3.1

Not everyone may be familiar with Microsoft® Windows™. The following section explains some basic aspects of Windows™. It is not meant to be an in-depth treatise: the Microsoft® documentation should be consulted for more details. If you are already familiar with Windows™, you can easily skip this section. If you have not used Windows™ before, we highly recommend Microsoft's on-line Windows™ tutorial. The tutorial can be found under Help.

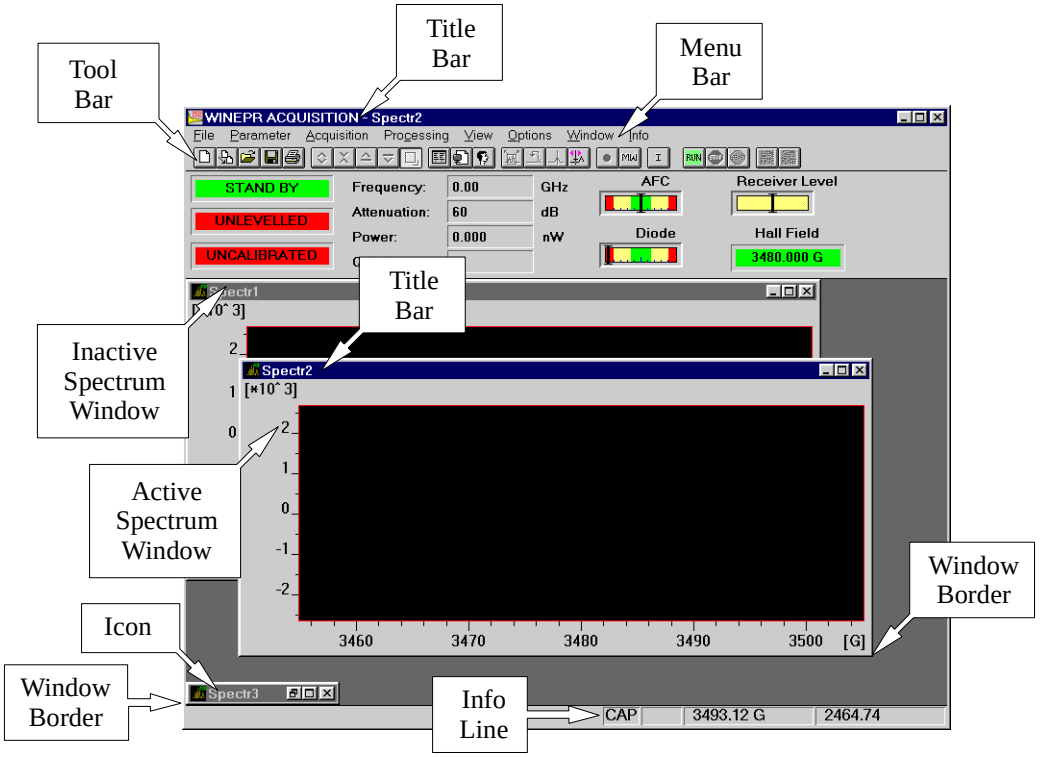


Figure 3-2 The parts of an applications window.

Application Window

All Windows™ programs operate in an application window. (See Figure 3-2.) WIN-EPR Acquisition displays all its commands and spectra in the application window.

Spectrum Window

Acquired spectra are displayed in a spectrum window. You may have multiple spectrum windows open at the same time, however, only one is active at a time. The active spectrum window is the one upon which operations will be performed. Each spectrum window has its own set of instrument parameters. The parameters viewed or edited in the menus correspond to those of the active spectrum window. With default Microsoft® Windows™ colors, the active windows have a blue title bar and the inactive windows have grey title bars. You activate a window by clicking it while the cursor is in the window. Spectrum windows may also be minimized to an icon at the bottom of the application window.

Title Bar

The bar at the top of a window is the title bar. Both the WIN-EPR application window and the spectrum windows have title bars. It shows the name of the application or of the spectrum window. The color of the title bar indicates whether a window is active or not. (See above.) By clicking and dragging the title bar, the window may be moved.

Other elements of the title bar are as follows.

Maximize Button

A click on the maximize button of the application window expands it to fill the entire screen. Clicking on the maximize button of the spectrum window expands it to fill the entire area of the application window. You may restore the window to its original size by clicking the restore button.

Minimize Button

Clicking on the minimize button of the application window shrinks the window and places it on the task bar. A click on the minimize button of the spectrum window shrinks it to an icon at the bottom of the application window.

Restore Button A click on the restore button returns the window to its previous size and locations. This button reverses the effect of using the maximize button.

Exit Button A click on the exit button closes the window.

Control Menu icon Double-clicking this icon closes the window. The appearance of the icon depends on the window or program you open. A single mouse click opens a drop-down menu. Consult your Microsoft® Windows™ documentation for further information regarding the commands in the menu.

Menu Bar The horizontal bar near the top of the application window is the menu bar. It displays the names of the available pull-down menus. Choose the desired menu by clicking on it with the left mouse button. The menu consists of a collection of commands. You choose a command by clicking on it with the left mouse button.

Tool Bar The horizontal bar below the menu bar is the tool bar. It displays buttons to execute the most commonly used commands. Clicking on a button performs the command.

Info Line The info line is a bar at the bottom of the application window. The left corner displays messages regarding the presently selected command or the status of the program. The two boxes next to NUM display values of cursor positions when the appropriate options are active.

Window Border The perimeter of the window is the window border. When the cursor is placed anywhere on the window border, a double arrow replaces the regular cursor. If you click and drag, the window may be resized to the desired size. If you drag a corner, the two sides that form the corner are resized simultaneously.

Dialog Boxes 3.1.1

Many commands open a dialog box. (See Figure 3-3.) The dialog box allows you to enter required input for the acquisition. What follows is a description of the basic elements of a dialog box and how to use them.

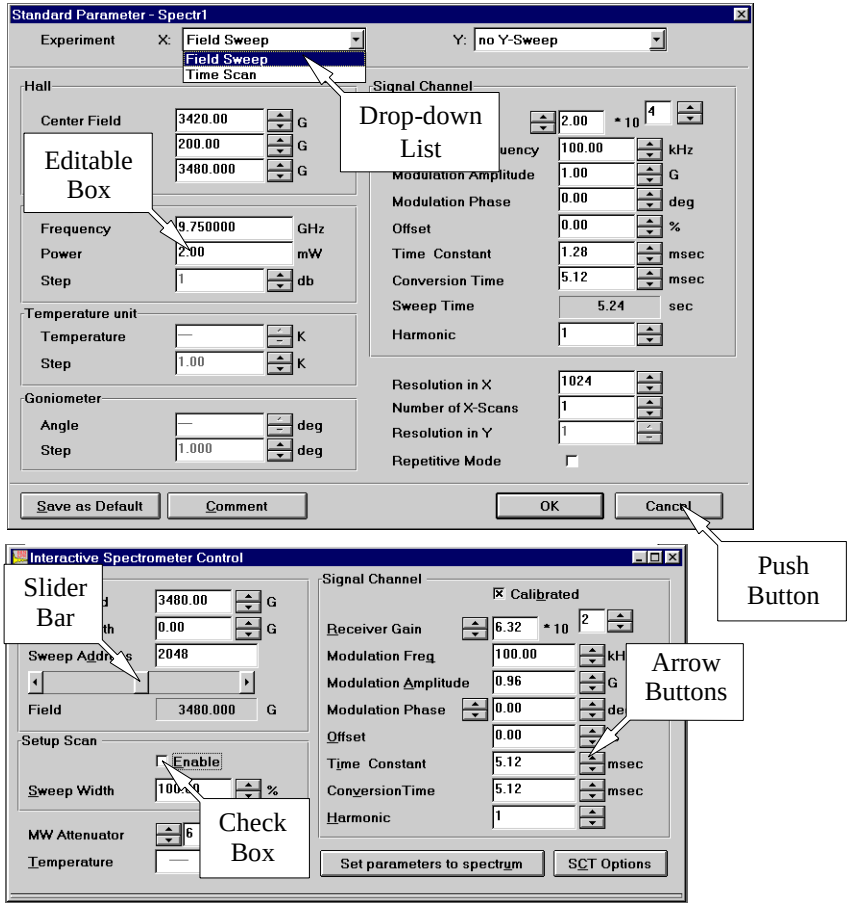


Figure 3-3 The parts of a dialog box.

Editable Box

The editable box is a plain box with a white background. As the name suggests, you may edit the value in the box. It is used for the input and display of quantities that are not restricted to specific values but may have a continuum of values such as the center field. After a click with the left mouse button in the text of the box, an insertion marker appears (a vertical line). Any text (or numbers) you type are inserted after the insertion marker. Several characters may be selected or highlighted simultaneously by clicking and dragging over the desired text. Any typed text replaces the highlighted text. The selected text may also be deleted by pressing the Del key. The left and right arrow keys of the keyboard moves the insertion marker left and right. Keeping the keys pressed repeats the action automatically.

Drop-down List

This input method is used for parameters that have a limited number of options or choices. After clicking on the downward pointing arrow next to the box, the allowed values appear in a drop-down list. The presently active option is highlighted. The highlighted choice is changed by pressing the up and down arrow keys of the keyboard. You may also select the desired choice by clicking the value with the left mouse button. The drop-down list then disappears with the newly selected value or option displayed in the box.

Check Box

The check box acts like a toggle. When clicked, the action turns the option on or off. A cross mark in the box indicates an on (or active) state.

Push Button

A push button will execute a command when you click it with the left mouse button. The command, such as OK or Cancel is displayed in the center of the button.

Arrow Buttons The arrow buttons are used to change a variable in a discrete step-wise fashion. If the box has a white background, the values may be edited as in an editable box. Clicking the up or down arrow button increases or decreases the parameter with a fixed step size. For example, the step size for modulation amplitude is 0.1 Gauss. Keeping the mouse button pressed repeats the action automatically. If the background of the box were gray, the up and down arrows next to the box move you through the allowed values for the variable sequentially. You are then not able to edit the values.

Slider Bar The slider bar is used to vary a parameter continuously between its allowed limits. For example, it is used to vary the microwave source frequency from 9.1 to 9.9 GHz. Clicking to the left or right of the square acts as a coarse adjustment while clicking the left or right arrows allows fine adjustments. Keeping the mouse button pressed repeats the action automatically. The value of the parameter is indicated graphically by the rectangle to supply you with visual feedback. The parameter may be varied as well by clicking and dragging the square.

Scroll Bar The scroll bar looks like a slider bar but functions differently. It is used to view entries in a list. For example, it is used in the Save As dialog box to choose subdirectories. Clicking the up or down arrows scrolls the list up and down. Keeping the mouse button pressed repeats the action automatically. The position of the viewed entries in the list is indicated graphically by the square. The list may be scrolled as well by clicking and dragging the square.

OK Button This button returns you to the original window or dialog box when clicked. All the changes made in the dialog box are set.

Cancel Button This button returns you to the original window or dialog box when clicked. All changes made in the dialog box are canceled.

Turning the Spectrometer On 3.2

If you are not sure how the electric power is connected, consult your local instrument or facilities manager.

1. **Turn on the power for the system.** How you do this depends on how the electric power was hooked up when the spectrometer was installed. Most likely you will activate the switch on the breaker box for the spectrometer. Breaker boxes are usually mounted on the wall. Consult the local instrument or facilities manager if you are not sure where the breaker box is.

2. **Turn on the power for the console.** The power switch for the console is located in the lower right corner of the back of the console. (See Figure 3-4.) The LED's on the various units in the console will light up.

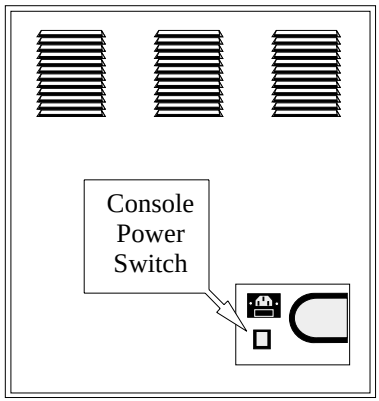


Figure 3-4 The location of the console power switch.

Make sure you do not have a floppy disk in the floppy disk drive when you turn the PC on.

3. **Turn on the power for the computer.** The details of turning on the computer depend on the type of the PC you have. Consult your PC user's manual.

4. **Turn on the tap water for cooling.** There are usually two valves, one for the supply and one for the return (or drain). Consult the local instrument or facilities manager if you are not sure where the valves are.

5. **Identify your power supply.** There are two types of power supplies and you must identify which type you have. (See Figure 3-5.) The small ER 080/081 power supplies, are located directly below the console and are accessed by opening the glass door. If you have this type of power supply, go to Step 6. The Bruker ER 082/083/085/086 magnet power supplies are larger. They

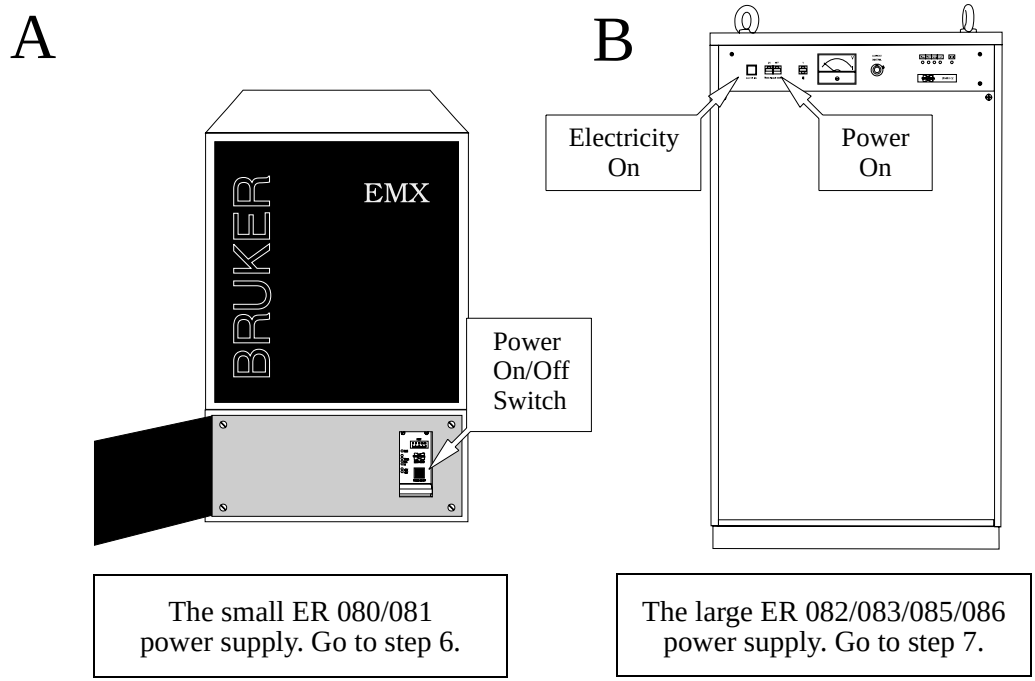


Figure 3-5 Two types of power supplies.

are not under the console but free standing. Skip to step 7 for instructions on turning your power supply on.

6. **Turn on the heat exchanger and magnet power supply. (Instructions for Small Power Supplies).** If you have a heat exchanger, (Not all systems require a heat exchanger.) you must first turn it on by activating the power switch. (See Figure 3-6.) To turn the power supply on, push the POWER ON/OFF button. (See Figure 3-5 A.) Step 8.

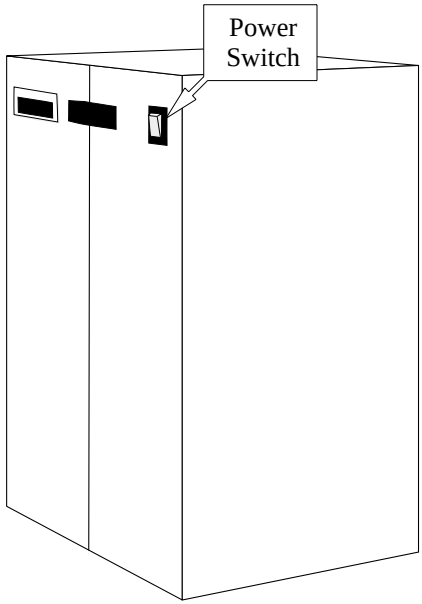


Figure 3-6 The location of the heat exchanger power switch.

7. **Turn on the heat exchanger and magnet power supply. (Instructions for Large Power Supplies)** On systems with large power supplies, (See Figure 3-5 B.) you need to first press the ELECTR. ON button and then the POWER ON button. Pressing the POWER ON button also starts the heat exchanger. If not, make sure that the power switch on the heat exchanger is activated. (See Figure 3-6.)

8. **Verify the water flow.** Make sure that the valves controlling the flow of water to the microwave bridge are open. The valve handles should be parallel to the hose. You can find them on either the left side or right side of the base of the magnet. (Follow the black water lines coming from the back of the bridge.) Make sure that the water lines are properly installed. Water coming out of the back of the bridge indicates a loose coolant tubing connector. If this happens, turn off the heat exchanger and power supply and tighten the leaky connector. Condensation on the magnet coils may indicate an improperly adjusted thermostat in the heat exchanger. If the magnet power supply shuts itself off, the thermostat may not be set properly or you do not have sufficient cold water flowing through the heat exchanger.

9. **Start the WIN-EPR Acquisition Application.** You should have Microsoft® Windows™ already running. Consult your Windows™ documentation for details. Locate the task bar on the desk top. (See Figure 3-7.) The position of the bar can be on the top, bottom, left, or right edge depending on how Windows™ is set up. Point to or click on Start and a drop down list will appear. Point to or click on Programs a cascading menu will appear. Point to or click on WIN-EPR and then click on Acquisit to start the program. The program will then initialize all the modules of the EMX spectrometer. You can also double click the Acquisit shortcut icon to start the program. If the

Acquisit shortcut has not been set up, consult your Windows™ documentation to learn how to set up a shortcut.

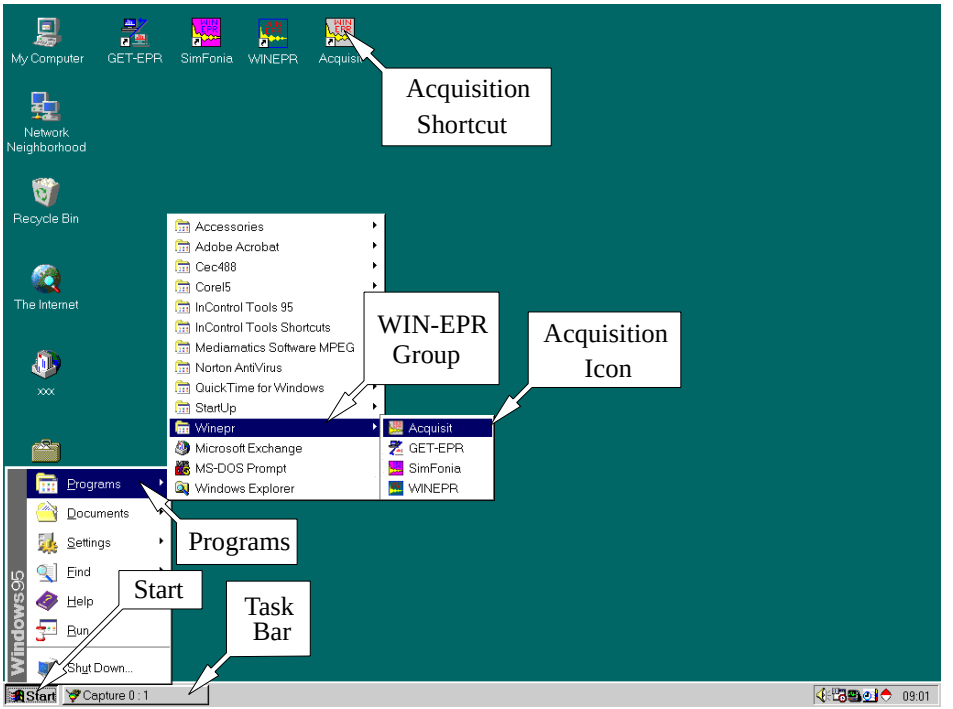


Figure 3-7 Starting the Win-EPR Acquisition Application.

10. **Install an EPR cavity if there is not one presently installed.** The instructions in this chapter assume you are using a properly installed Bruker ER 4102ST cavity. If there is no cavity installed, seek the assistance of a knowledgeable EPR colleague or refer to Chapter 5 to learn how to install a cavity.

11. **Proceed to Section 3.3**.

Removing and Inserting Samples 3.3

1. **Open the Microwave Bridge Control dialog box.** If this window is not already open, click the button labeled MW in the tool bar. The MW button toggles the dialog box open and closed. The microwave bridge control dialog box will then appear. (See Figure 3-8.)

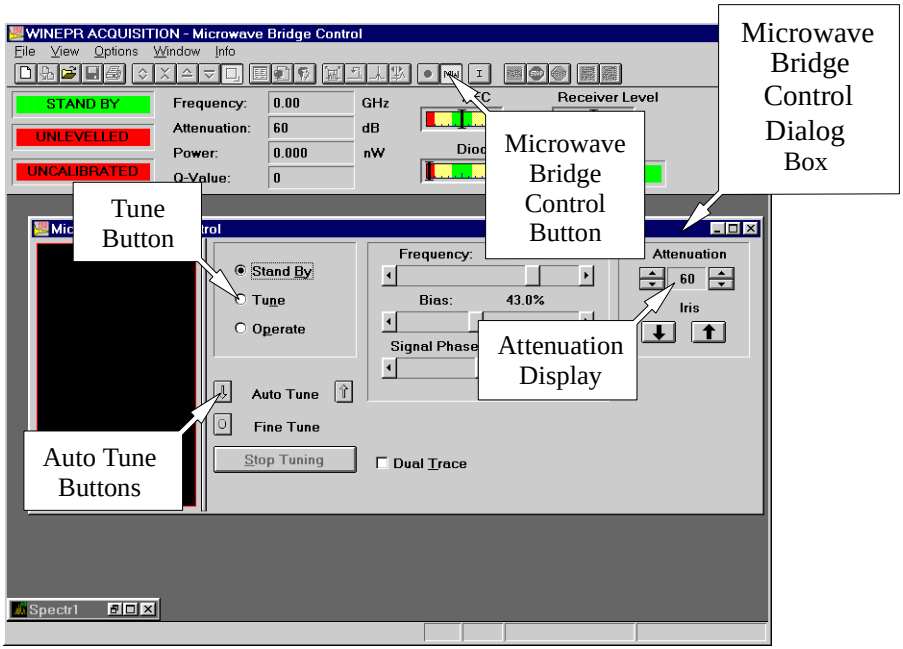


Figure 3-8 The Microwave Bridge Control dialog box.

2. **Switch the microwave bridge to Tune.** There are three states or modes for the microwave bridge, Stand By, Tune, and Operate. When you turn on your

If a klystron bridge does not switch to Tune and the Stand By indicator is red, wait a minute. There is a time delay of approximately three minutes between the time the console is turned on and the time the klystron can be turned on. This allows the klystron to warm up sufficiently.

Take care if you are wearing an analog (mechanical) watch. The magnetic field in the air gap of the magnet is sufficiently strong to magnetize your watch! Therefore, to avoid damage to your watch, remove your watch before putting your hands in the magnet air gap.

spectrometer, it should be in Stand By. This is indicated by Stand By (in green color) appearing in the microwave bridge controller menu. (See Figure 3-8.) If you have been acquiring spectra already, your bridge will probably be in Operate. Click the Tune button in the dialog box to switch the bridge to Tune.

3. **Set the microwave attenuator to 25 dB.** The microwave attenuation is set by clicking the arrow boxes on either side of the attenuation display in the dialog box. (See Figure 3-8.)

4. **Remove the sample.** If there already is a sample in the cavity, remove it. Loosen the top collet nut (You do not need to remove it.) and carefully remove the sample from the cavity. Pulling the sample tube out as straight as possible prevents you from destroying your valuable samples. (See Figure 3-9, Figure 3-10, and Figure 3-11 for details.)

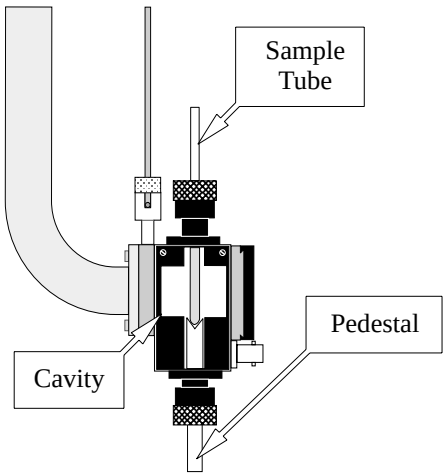


Figure 3-9 Cutaway view of a Bruker ER 4102ST cavity.

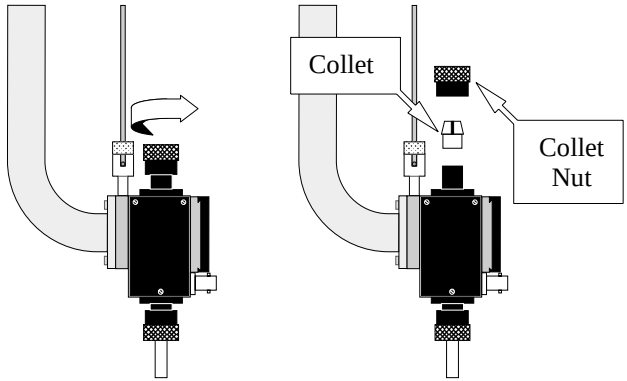


Figure 3-10 Loosening of the collet nut and removal of the collet.

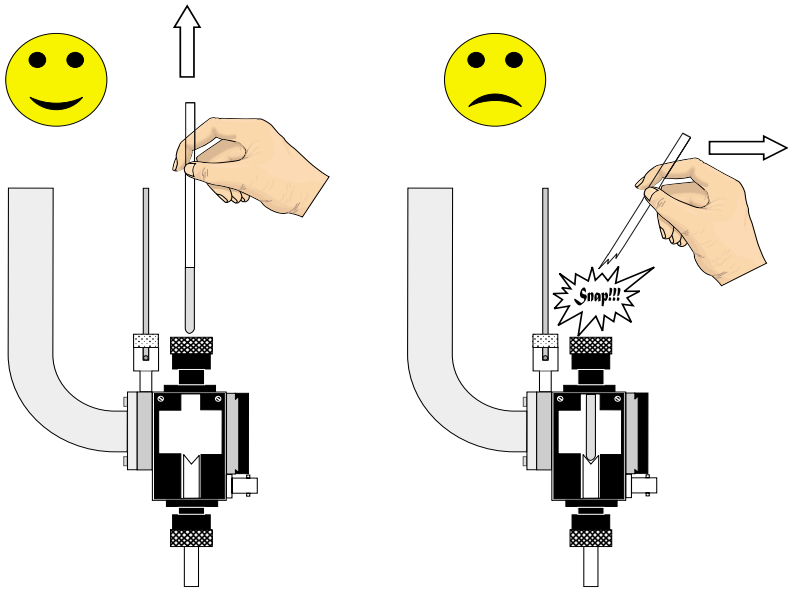


Figure 3-11 Right and wrong technique for removing a sample.

If this is your first time operating an EMX spectrometer, we recommend that you use the strong pitch sample supplied with your instrument. Our instructions in this chapter are based on using this sample.

5. **Clean the sample tube to be inserted into the cavity.** It is vital to avoid contaminating the microwave cavity as a paramagnetic contaminant may produce spurious EPR signals or distorted base lines. Wiping the outside of the sample tube with tissue paper is usually adequate.

6. **Insert the sample tube carefully into the cavity.** (See Figure 3-9 and Figure 3-10.) Make sure you have the appropriate collet size for your sample tube size. The tube should be slightly loose before you tighten the collet nut. The bottom of your sample should rest in the indentation on the pedestal. This ensures that your sample is centered horizontally. The height can be adjusted by loosening the bottom collet nut and moving the pedestal up and down. Tighten the top collet nut to firmly hold the sample tube in place and the bottom collet to firmly hold the pedestal.

Make sure that the pedestal is not in the cavity, as it can give an EPR signal.

7. **Tune the cavity.** Details on this procedure are given in the next section.

Tuning the Microwave Cavity and Bridge 3.4

1. **Open the Microwave Bridge Control dialog box.** If this window is not already, open click the button labeled MW in the tool bar. The MW button toggles the dialog box open and closed. (See Figure 3-12.)

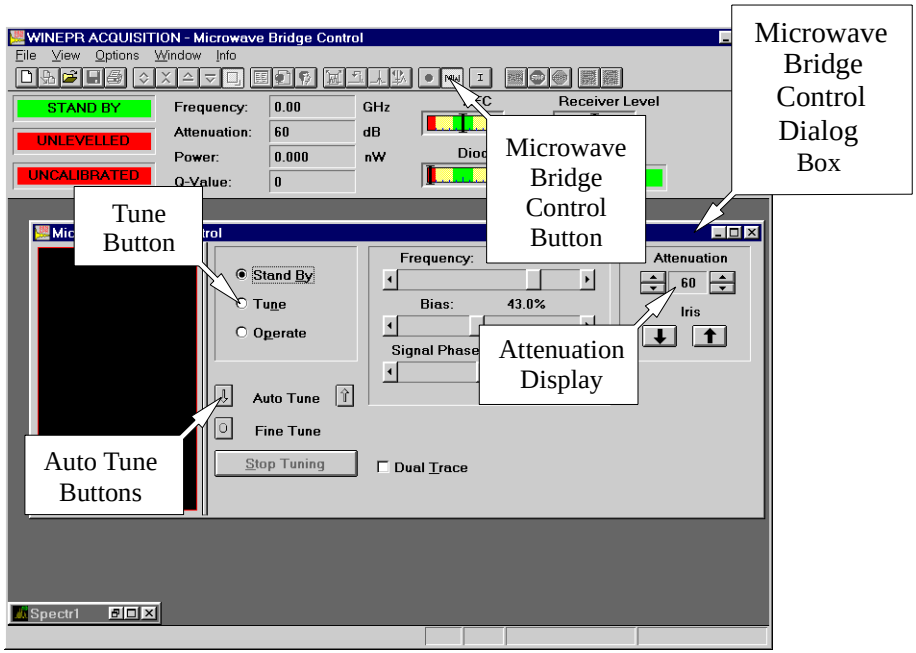


Figure 3-12 The Microwave Bridge Control dialog box.

If a klystron bridge does not switch to Tune and the Stand By indicator is red, wait a minute. There is a time delay of approximately three minutes between the time the console is turned on and the time the klystron can be turned on. This allows the klystron to warm up sufficiently.

It is vital to avoid contaminating the microwave cavity as a paramagnetic contaminant may produce spurious EPR signals or distorted base lines.

2. **Switch the microwave bridge to Tune.** There are three states or modes for the microwave bridge, Stand By, Tune, and Operate. When you turn on your spectrometer, it should be in Stand By, which is indicated by Stand By appearing in the Microwave Bridge Control menu. (See Figure 3-12.) If you have been acquiring spectra already, your bridge will probably be in Operate. Click the Tune button in the dialog box to change to Tune.

3. **Set the microwave attenuator to 25 dB.** The microwave attenuation is set by clicking the arrows on either side of the attenuation display in the dialog box. (See Figure 3-12.)

4. **Clean the sample tube to be inserted into the cavity.** Wiping the outside of the sample tube with tissue paper is usually adequate.

5. **Insert the sample tube carefully into the cavity.** If you have inserted the sample already, proceed to Step 6., otherwise see Section 3.3 for details on how to do this.

6. **Tune the bridge and cavity.** Pressing either of the arrow buttons on both sides of Auto Tune starts the automatic tuning procedure. (See Figure 3-12.) The up arrow starts by scanning the microwave frequency up in search of the cavity dip (or frequency where the cavity resonates). The down arrow starts by scanning the microwave frequency down in search of the cavity dip. If you are not sure if the search should start up or down, do not worry. The frequency will be scanned until its limit is reached and then scan in the other direction until the cavity dip is found. The Auto-Tune routine adjusts the frequency, phase, and bias of the bridge and the coupling (matching) of the cavity. If there is an error message, there may be something wrong with the instrument. Notify the facility manager or see Chapter 7 for trouble shooting.

7. **Close the Microwave Bridge Control dialog box.** Click the bridge controller button (the button labeled MW) in the tool bar. The MW button toggles the dialog box open and closed. The Microwave Bridge Control dialog box will then disappear. (See Figure 3-12.)

8. **Proceed to the next section to learn how to acquire spectra.**

Acquiring Spectra 3.5

1. **Follow the instructions of Section 3.2 through Section 3.4.** You should have the spectrometer turned on, a Bruker ER 4102ST standard cavity installed with a strong pitch sample in it, and the microwave bridge and cavity tuned.

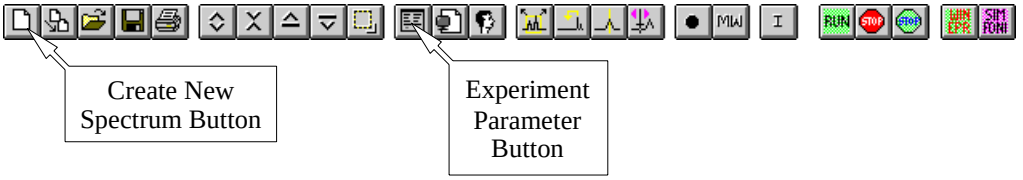


Figure 3-13 The Experiment Parameter and Create New Spectrum buttons.

2. **Create a new spectrum window, if needed.** If you have just started the Acquisition program, it automatically presents you with an empty spectrum window containing default parameters. If there is no empty spectrum window, create one by clicking on the Create New Spectrum button in the toolbar. (See Figure 3-13.)

3. **Open the Experiment Parameter dialog box in order to check the parameters.** If this window is not already open, click its button in the tool bar. (See Figure 3-13.) The button toggles the dialog box open and closed.

4. **Check the experiment type.** Check to see if Experiment X: is set to Field Sweep. (See Figure 3-14.) If it is set to Time Sweep, change it to Field Sweep. Experiment Y: must be set to No Y-Sweep.

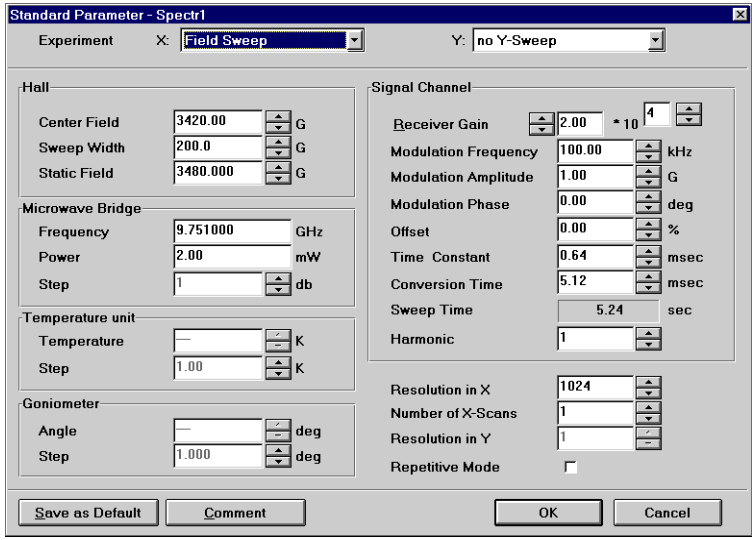


Figure 3-14 The Experiment Parameter dialog box.

5. **Check the field sweep parameters.** The parameters for the field sweep are listed in the Hall (for Hall effect field controller) section. Edit the values of the parameters so they correspond to the ones in Figure 3-14. The Static Field parameter is not used in this experiment; therefore, you do not have to edit its value.

6. **Check the Signal Channel Parameters.** Edit the values of the parameters so they correspond to the ones in Figure 3-14.

7. **Set the Microwave Power.** Enter a value of 2.0 in the Microwave Power parameter box.

8. **Close the Experiment Parameter dialog box.** Click on the OK button and the dialog box will then disappear. Cancel exits the dialog box without saving the changes.

Changing a parameter followed by clicking OK wipes out the spectrum in the active window. The software is designed to maintain a one-to-one correspondence between a spectrum and parameters.

9. **Open the Experiment Options dialog box in order to check the options.** If this window is not already open, click its button in the Tool Bar. (See Figure 3-15.) The button toggles the dialog box open and closed. The Experiment Options dialog box will then appear. (See Figure 3-16.)

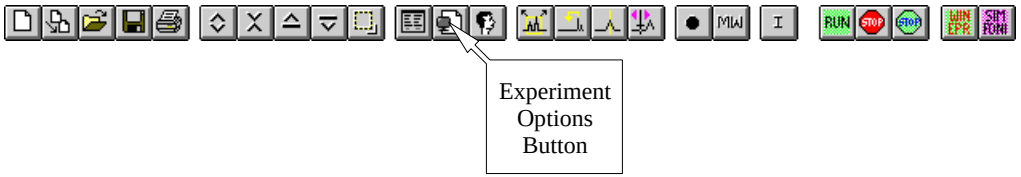


Figure 3-15 The Experiment Options button.

10. **Check the Microwave Settings.** Click and select the Set option. With this option, the microwave power we entered in Step 7. will be the one used to acquire the spectrum.

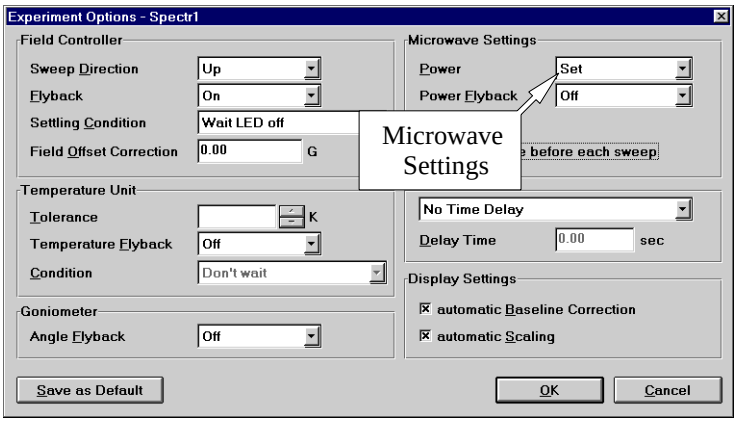


Figure 3-16 The Experiment Options dialog box.

11. **Close the Experiment Options dialog box.** Click on the OK button and the dialog box will then disappear. Cancel exits the dialog box without saving the changes.

Figure 3-17 The Location of the RUN Button.

12. **Acquire a spectrum.** Click the RUN button to start an acquisition. (See Figure 3-17.) If you have a similar spectrum like the one in Figure 3-18, congratulations! You have successfully acquired an EPR spectrum. You may notice that the EPR line is not nicely centered. The next step will help you center your spectrum.

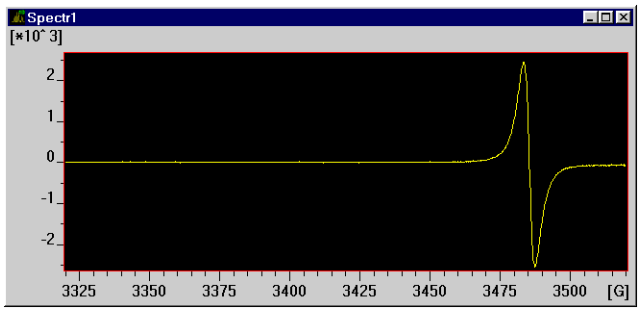


Figure 3-18 An EPR spectrum of strong pitch.

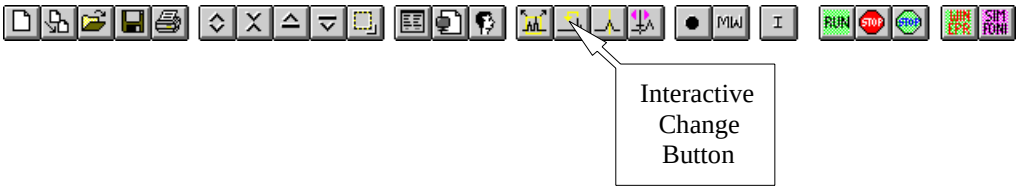


Figure 3-19 The Interactive Change of Center Field Parameter button in the Tool Bar.

13. **Center a spectrum.** To interactively set the center field, click the Interactive Change of Center Field Parameter button in the Tool Bar. (See Figure 3-19.) Clicking this button creates a marker (vertical line) in the spectrum window that moves with the cursor. (See Figure 3-20.) Place the cursor where you would like the

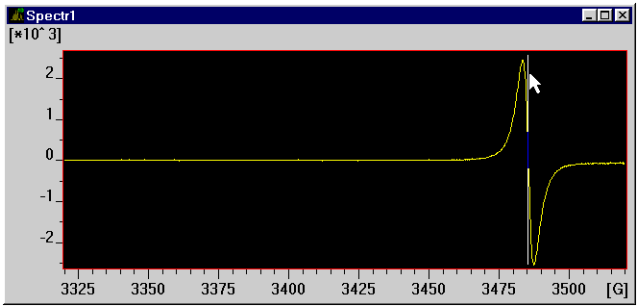


Figure 3-20 The center field marker.

center field to be and click with the right mouse button. This action replaces the center field value with the magnetic field position of the marker. To acquire the spectrum with the new center field, click on the RUN button in the

tool bar. The newly acquired spectrum will then be nicely centered. (See Figure 3-21.)

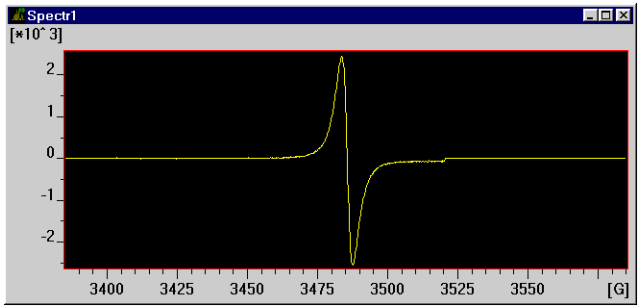


Figure 3-21 A centered spectrum.

14. **Enter a comment.** If this window is not already open,

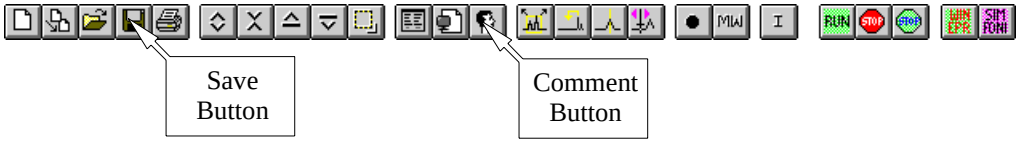


Figure 3-22 Buttons for saving files and comments.

Comments are very useful because you can keep track of such things as sample preparation details.

click its button in the tool bar. (See Figure 3-22.) The button toggles the dialog box open and closed. The Comment dialog box will then appear. (See Figure 3-23.) Enter a comment regarding your spectrum. The comments supplement the information which is included in the spectrum parameters. If you wish, you may enter your name in the operator box. Operator names are useful for keeping track of who acquired the spectrum. Click on the OK button and the dialog box will then disappear. Cancel exits the dialog box without saving the changes.

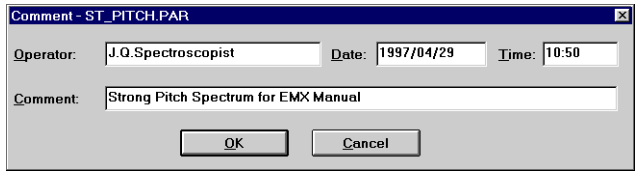


Figure 3-23 The Comment dialog box

15. **Save the spectrum to a disc.** After you have acquired a spectrum, you may save it in any folder. Click on the Save button in the tool bar. A dialog box appears that lets you choose a filename, a destination folder, and a destination disk drive. (See Figure 3-24.) The spectrum to be saved is the spectrum that is presently active. To select the appropriate disk drive, click on the arrow on the Drives: selector. To select the appropriate folder, click on the appropriate paths in the Folders: selector. The spectrum filename is selected by typing the filename in the File Name selector. Clicking OK saves the spectrum on the hard disk or diskette. Cancel exits the dialog box without saving the spectrum.

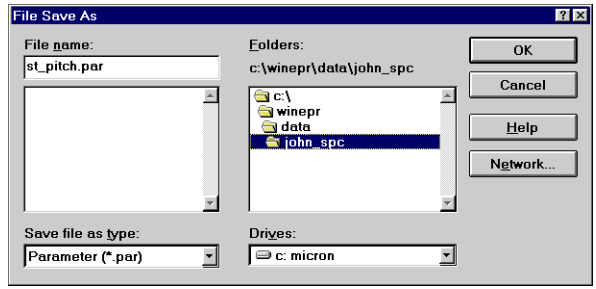


Figure 3-24 The File Save As dialog box.

If the chosen filename were already used by another file, a warning box gives you the opportunity to decide whether to replace the existing file with the present spectrum. (See Figure 3-25.) Pressing No cancels the save process and allows you to select another name or folder.

Figure 3-25 Warning dialog box for overwriting files.

Is your printer on line?

16. **Prepare to print the spectrum.** Turn the printer on. Make sure a sheet of paper is loaded. Refer to your printer documentation for details.

17. **Select the Output Formatting.** Click on the File menu bar and then click Output Formatting. A cascading menu will appear as shown in Figure 3-26. Select Spectrum + Parameters by clicking on it. A checkmark next to it indicates the option is active. Both the spectrum as well as the instrumental parameters will be displayed when you print the spectrum.

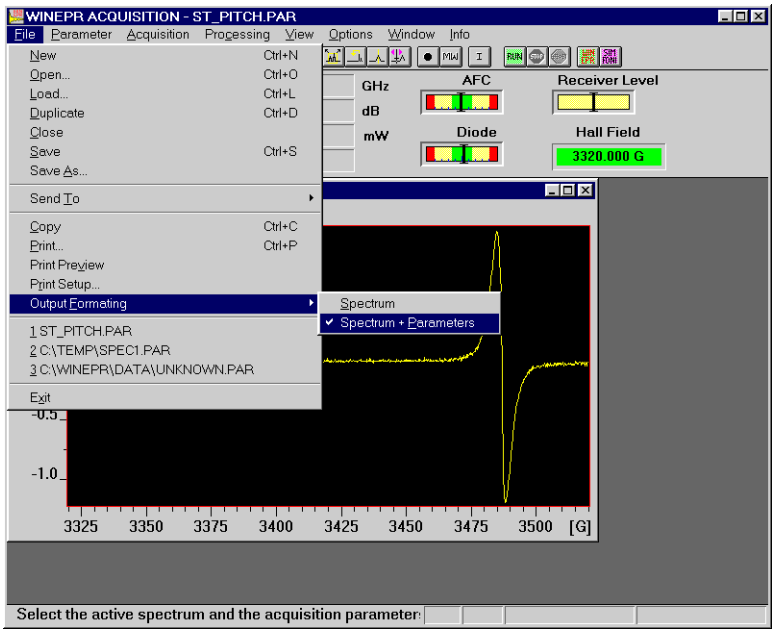


Figure 3-26 Selection of the Output Formatting.

18. **Print the spectrum.** Click on the Print button in the tool bar. (See Figure 3-27.) A dialog box will then appear in which you enter the desired options and print your spectrum. (See Figure 3-28.) Consult your printer and Windows™ documentation for further details regarding the options. Clicking OK starts the document printing and

Figure 3-27 Print button.

closes the dialog box. Clicking Cancel closes the dialog box without printing anything. The output from the printer will look similar to Figure 3-29.

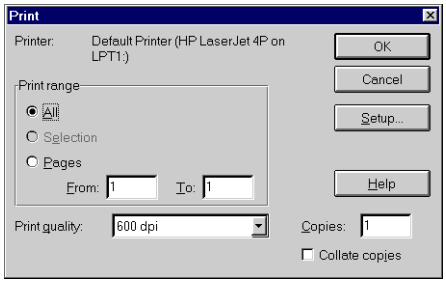


Figure 3-28 The Print dialog box.

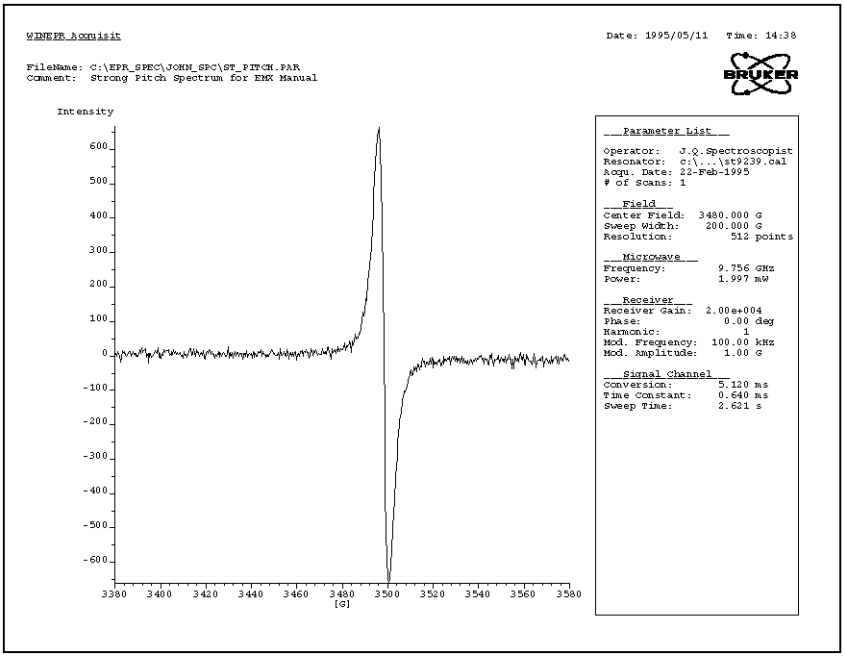


Figure 3-29 Typical output from the printer.

Turning the Spectrometer Off 3.6

1. **Open the Microwave Bridge Control dialog box.** If this window is not already open, click its button (the button labeled MW) in the tool bar. The button toggles the dialog box open and closed. The Microwave Bridge Control dialog box will then appear. (See Figure 3-30.)

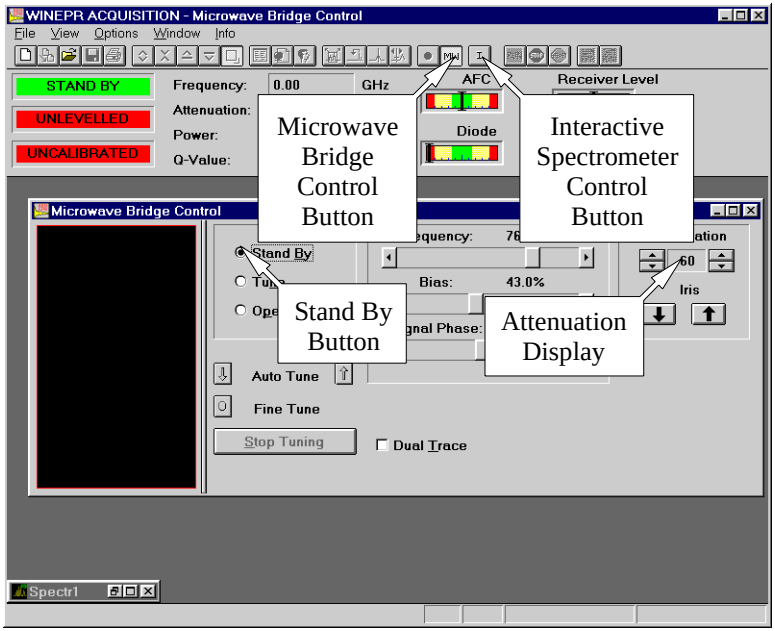


Figure 3-30 The Microwave Bridge Control dialog box.

2. **Switch the microwave bridge to Stand By.** (See Figure 3-30.) Click the Stand By button in the dialog box to change to Stand By. The microwave attenuator will be automatically set to 60 dB.

3. **Close the Microwave Bridge Control dialog box.** Click the bridge controller button (the button labeled MW) in the tool bar. The button toggles the dialog box open and closed. The Microwave Bridge Control dialog box will then disappear.

4. **Remove the sample from the cavity.** See Section 3.3 for details on how to do this.

5. Cover the upper collet or insert a blank collet plug.

Exit the software first before turning the PC off.

6. **Exit the WIN-EPR Acquisition program.** Click on the File menu bar and then click Exit. If there are any unsaved spectra, you will be asked if you wish to save them. It is important to exit the software in an orderly manner (*i.e.* don't just turn the computer off before exiting the software) because many instrument parameters are set to specific values for a safe shut-down of the spectrometer.

7. **Identify your power supply.** There are two types of power supplies and you must identify which type you have. (See Figure 3-31.) The small ER 080/081 power supplies, are located directly below the console and are accessed by opening the glass door. (See Figure 3-31 A.) If you have this type of power supply, go to Step 8. The Bruker ER 082/083/085/086 magnet power supplies are larger. They are not under the console but free standing. (See Figure 3-31 B.) If you have this type of power supply, skip to Step 9. for instructions on turning your power supply off.

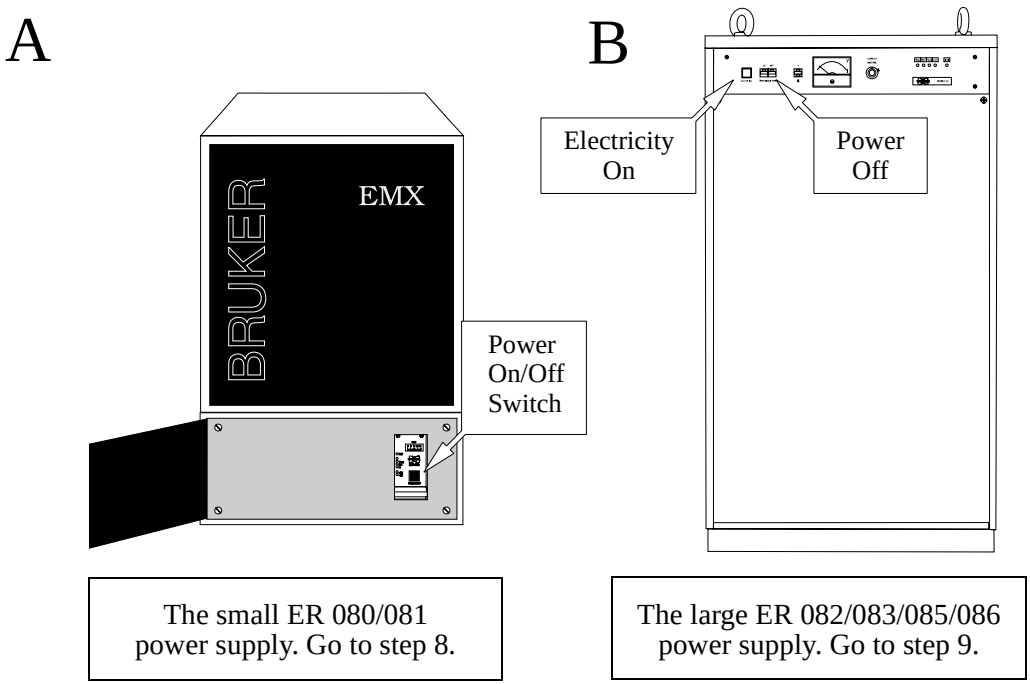


Figure 3-31 Two types of power supplies.

8. **Turn off the heat exchanger and magnet power supply. (Instructions for Small Power Supplies).** Turn the power supply off by pushing the POWER ON/OFF button. (See Figure 3-31A.) If you have a heat exchanger, (Not all systems require a heat exchanger.) you must turn it off by pressing the power switch. (See Figure 3-32.) Skip to step 10 for instructions on turning the tap water off.

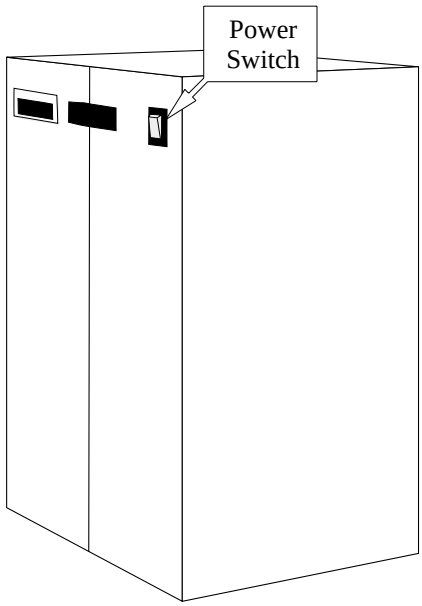


Figure 3-32 The location of the heat exchanger power switch.

9. **Turn off the heat exchanger and magnet power supply. (Instructions for Large Power Supplies)** On systems with large power supplies, the power supply is not under the console but free standing. (See Figure 3-31B.) You need to first press the POWER OFF button and then the ELECTR. ON button. Pressing the POWER OFF button also turns the heat exchanger off.

10. **Turn off the tap water for cooling.** There are usually two valves, one for the supply and one for the return (or drain). Consult the local instrument or facilities manager if you are not sure where the valves are.

If you have many power outages or electrical storms, it is a very good idea to shut off power to the spectrometer.

11. **Turn off the power for the console.** The power switch for the console is located in the lower right corner of the back of the console. (See Figure 3-33.)

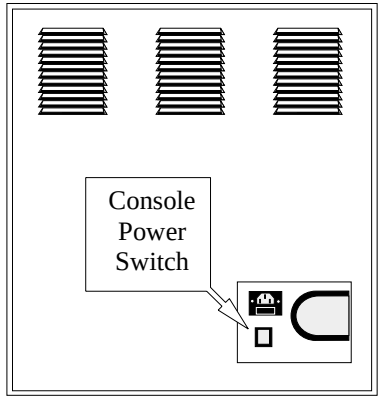


Figure 3-33 The location of the console power switch.

12. **Turn off the power for the system.** How you do this depends on how the electric power was hooked up when the spectrometer was installed. Most likely you will deactivate the switch on the breaker box for the spectrometer. Breaker boxes are usually mounted on the wall. Consult

the local instrument or facilities manager if you are not sure where the breaker box is.

A Brief EMX Tutorial 4

This chapter contains useful and helpful hints to get the most out of the WIN-EPR Acquisition software. In the previous chapter, we blindly followed many instructions to acquire a spectrum. Here is the opportunity to explore some of the features in a bit more depth. The tutorial is not meant to be an exhaustive treatise on all details of the spectrometer. Instead, it is a starting point from which you can explore the capabilities of the instrument on your own.

The first topic covers advice on spectrum windows such as how to keep things neat, transferring parameters, and zooming in on specific areas of a spectrum. The second topic describes stopping and starting acquisitions. Field sweep experiments and the adjustment of parameters are covered in the third topic. The fourth topic deals with time scans. Interactive adjustment of spectrometer parameters is described in the fifth topic. The sixth topic contains advice on options for controlling the microwave bridge. The chapter ends with advice on saving files and how to export spectra to WIN-EPR for more sophisticated post-processing.

Spectrum Windows 4.1

Keeping Things Neat 4.1.1

With an EMX spectrometer, you can generate a very large number of spectra very quickly. The spectrum windows can completely clutter your screen just as quickly. There are several ways to keep things neat. The first approach is to cascade or tile the windows to organize them better. The commands for performing these operations are found in the Window menu. Perhaps the best way to explain what each of these commands does is to look at the following examples. (See Figure 4-1, Figure 4-2, Figure 4-3.)

The Cascade command stacks all the open windows such that their title bars are all visible.

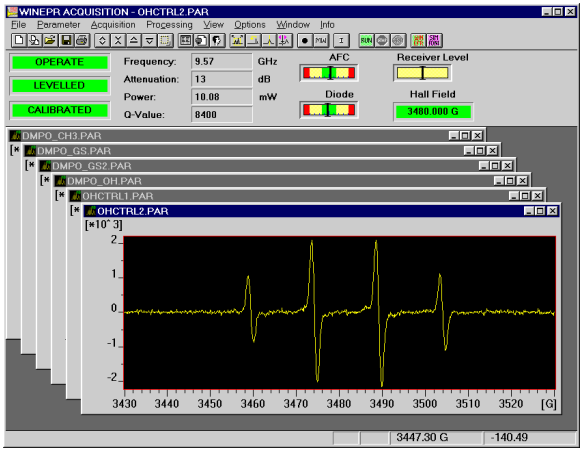


Figure 4-1 Cascaded spectra.

The Tile Horizontal command arranges all the open windows top to bottom such that they are all visible.

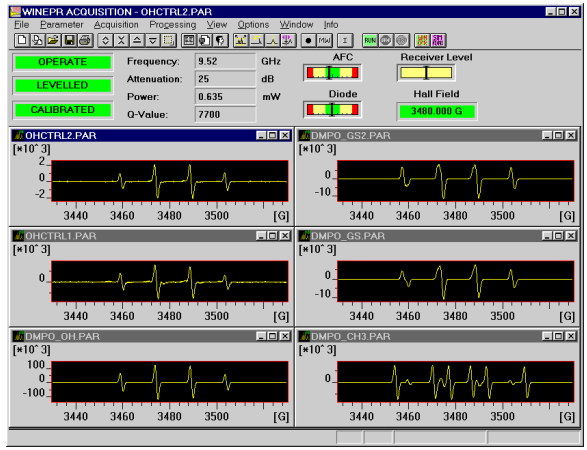


Figure 4-2 Horizontal tiling.

The Tile Vertical command arranges all the open windows side by side such that they are all visible.

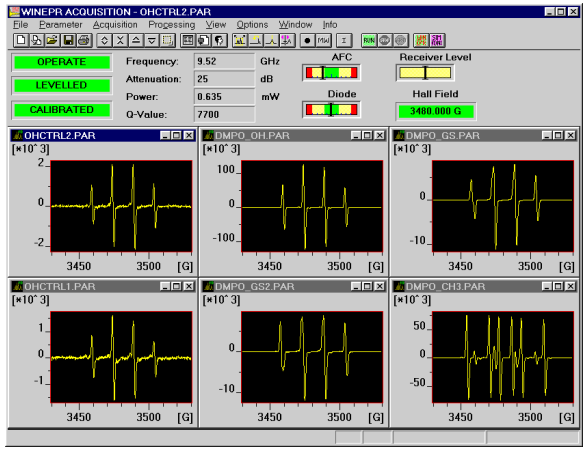


Figure 4-3 Vertical tiling.

A second approach to neaten the display is to iconize the spectrum windows so they require considerably less space on the screen. (See Figure 4-4.) You lose the ability to see the contents of a window immediately, however, you gain almost instantaneous access to many more spectra than with the previous approaches. The Arrange Icons command neatly organizes the spectrum windows at the bottom of the application window.

Icons allow quick access to many spectra in your application window.

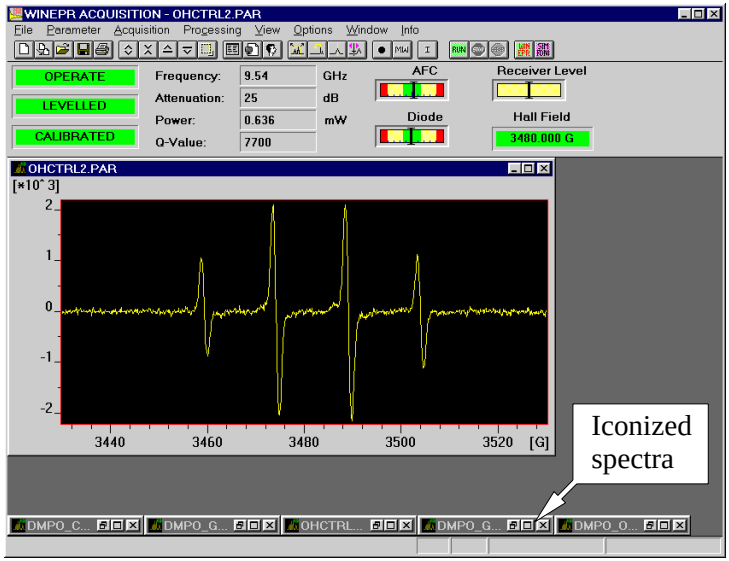


Figure 4-4 Iconized spectra.

Creating a New Spectrum Window 4.1.2

You may need to create a new window in which you can acquire a spectrum. Simply click the Create New Spectrum button in the tool bar. (See Figure 4-5.) A new spectrum window will appear having the default parameter set.

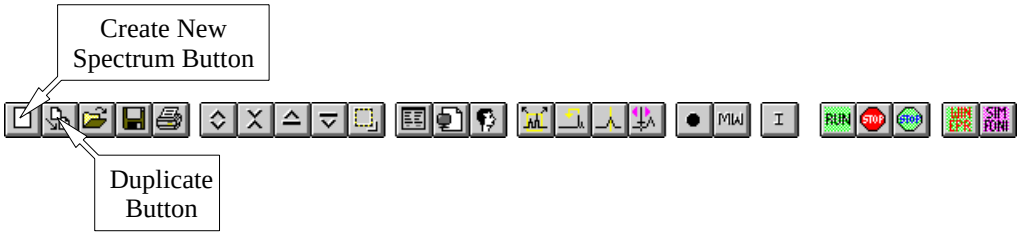


Figure 4-5 The Duplicate and Create New Spectrum buttons in the tool bar.

Transferring Parameters 4.1.3

In order to perform drag and drop functions, you have to turn off certain functions such as Zooming, Change Center Field and Sweep Width, Change Center Field, Change Static Field, and Interactive Receiver Level.

You may often need the same set of parameters to acquire a whole series of spectra. The parameters of one spectrum window can be easily transferred to another spectrum window. This is easy accomplished by using the Duplicate button in the tool bar. (See Figure 4-5.) Clicking this button creates a new spectrum window with parameters that are identical to the original active spectrum. You can also drag and drop an EPR spectrum and its parameters to another spectrum window. Click the spectrum window with the left mouse button and the pointer will change into a hand sign. Drag to the spectrum window where you want to copy and the pointer will change into a spectrum sign. Releasing the mouse button will copy the spectrum and the parameters to the window.

Resizing Spectrum Windows 4.1.4

You can fit more spectra in the application window if you resize the spectrum windows to a smaller size. Make sure they are large enough to see all their important details. Click and drag the window borders to change the size of the spectrum. You can still make them larger by clicking the Maximize button. Details are given in Section 3.1.

Zooming Spectra 4.1.5

You can zoom in on specific areas of a spectrum in two ways. The first way is to use the tools in the tool bar. (See Figure 4-6.) Spectra can be increased or decreased in size by factors of two by clicking the expand and contract buttons. Clicking on the offset arrows shifts the spectrum up and down. Note: these actions only affect the display, not the actual data set.

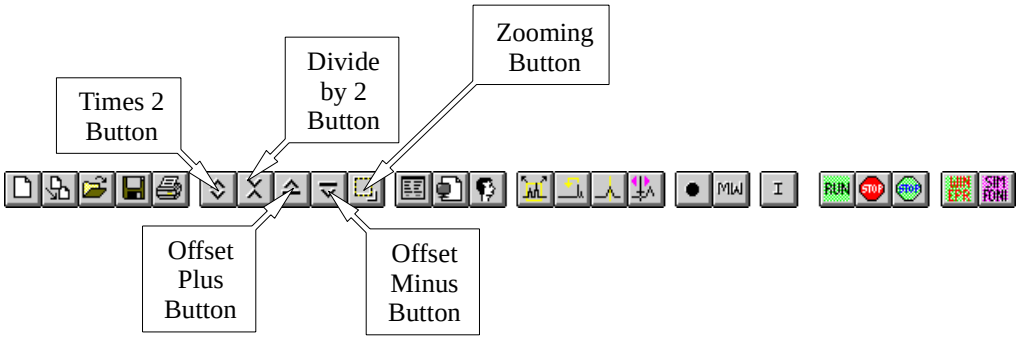


Figure 4-6 Some Display commands in the tool bar.

Times 2 Clicking the Times 2 command decreases the vertical display range by a factor of two. This corresponds to enlarging the vertical size of the spectrum by a factor of two. Pressing function key F5 from the keyboard has the same result.

Divide by 2 Clicking the Divide by 2 command increases the vertical display range by a factor of two. This corresponds to reducing the vertical size of the spectrum by a factor of two. Pressing function key F6 has the same result.

Offset Plus Clicking the Offset plus command shifts the spectrum upwards. Pressing function key F7 has the same result.

Offset Minus Clicking the Offset minus command shifts the spectrum downwards. Pressing function key F8 has the same result.

Reset All of the above operations may be reversed by clicking the right mouse button. This action resets the display so that the spectrum is entirely in the window. Pressing Ctrl + R has the same result.

A second manner to zoom spectra is to use the rectangular scaling option. Clicking on the Zooming button in the tool bar activates or deactivates this option. Click the left mouse button and drag the rectangle until it encompasses the region of interest. Click the right mouse button and the region of interest will then expand to fill the spectrum window. (See Figure 4-7.) When this option is active, clicking the left mouse button toggles the cursor between the lower right and upper left corner of the zoom rectangle. As you move the cursor, the position of the rectangle corner moves with the cursor. Clicking with the right mouse button expands the region encompassed by the rectangle to fill the whole window.

All the preceding zooming can be undone by clicking on the Reset command or clicking the right mouse button.

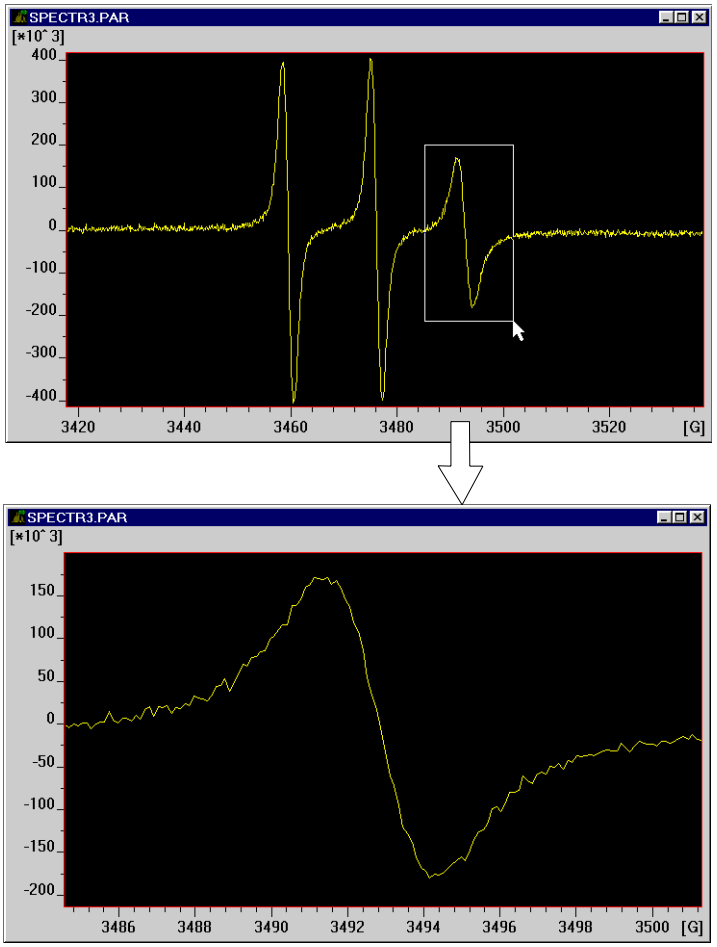


Figure 4-7 Rectangular zooming and its result.

Starting and Stopping Acquisitions 4.2

There are three handy buttons in the toolbar for starting and stopping acquisitions. The RUN button in the tool bar starts acquisitions. You can also start the acquisition by clicking Start Acquisition Run in the pull down menu of the Acquisition menu. Acquisitions may be stopped in two ways. The red STOP button stops the acquisition immediately. The green STOP button is used when signal averaging. It stops the acquisition only after the end of a field sweep or time sweep has been reached. (See Figure 4-8.)

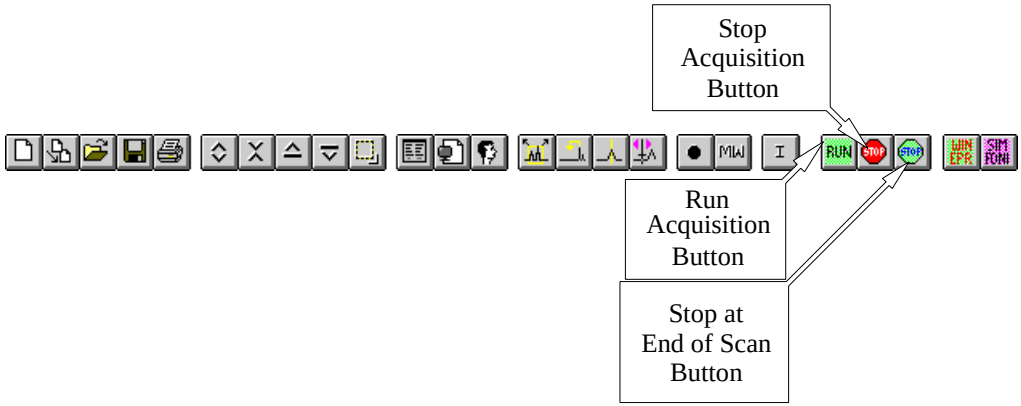


Figure 4-8 Start and stop commands in the tool bar.

Field Sweeps 4.3

Setting Parameters via Zooming 4.3.1

If you are searching for EPR signals from an unknown species, the most prudent approach to find signals is to make a very broad scan with the center field set to a value where you expect to see a signal. (See Section 6.1, Hints for Finding EPR Signals.) This approach maximizes the probability of finding a signal in your field sweep. If you are lucky, the EPR signals will already be nicely centered in the field sweep, most of the field sweep will contain EPR signals and not empty baseline, and the receiver gain will be set perfectly. Such luck rarely occurs! The Interactive Change of Center Field and Sweep Parameter button (Figure 4-9) helps you to achieve the desired results on your second attempt. The following procedure allows you to use a broad scan to optimize the center field, sweep width, and receiver gain so that you can acquire an aesthetically pleasing as well as meaningful spectrum.

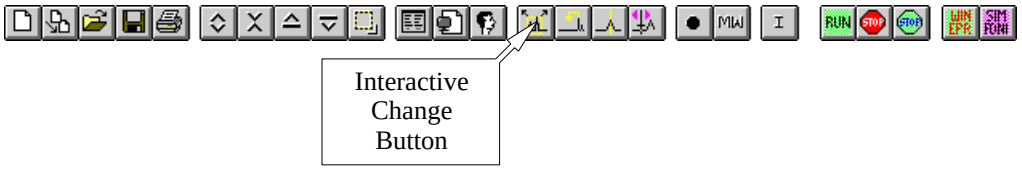


Figure 4-9 The Interactive Change of Center Field and Sweep Parameter button in the tool bar.

In Figure 4-10, we have used a broad field scan to find our EPR signal. Clicking the Interactive Change of Center Field and Sweep Parameter button in the tool bar creates a zoom rectangle in the spectrum window. This rectangle functions in the same manner as the rectangular scaling option described in Section 4.1.5. Surround the area that you would like to have in your spectrum and click the right mouse button. The first thing you will notice is that the selected region will be zoomed or expanded to fill the entire spectrum window. If you open the Experiment Parameter dialog box, you will also notice that the receiver gain, center field and sweep width have been adjusted such that the spectrum will fit in the spectrum window with optimal receiver gain.

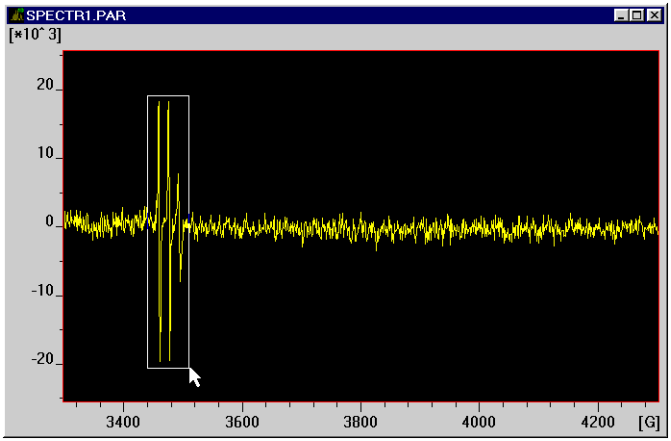


Figure 4-10 The zoom rectangle for interactive adjustment of parameters.

We are not done yet; the expanded spectrum does not have the same number of points as the original spectrum. You also need to acquire the spectrum with the newly optimized parameters.

Click on the RUN button in the tool bar. The spectrum will then be nicely centered as in Figure 4-11 after being acquired with the optimized center field, receiver gain, and sweep width.

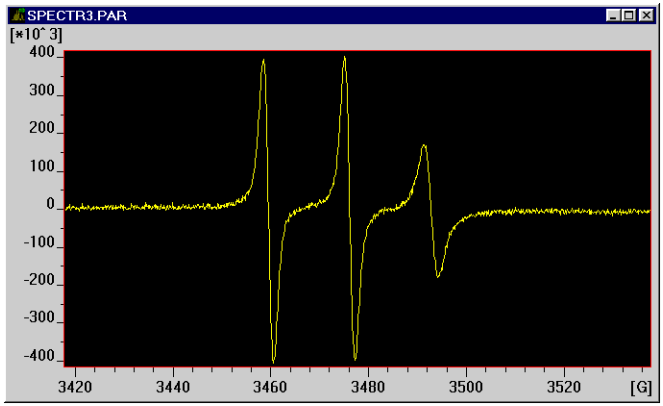


Figure 4-11 An optimized spectrum.

Setting Center Fields 4.3.2

Sometimes you may not have to change all the parameters such as receiver gain and sweep width: setting the center field may be sufficient. To interactively set the center field, click the Interactive Change of Center Field Parameter button in the tool bar. (See Figure 4-12.)

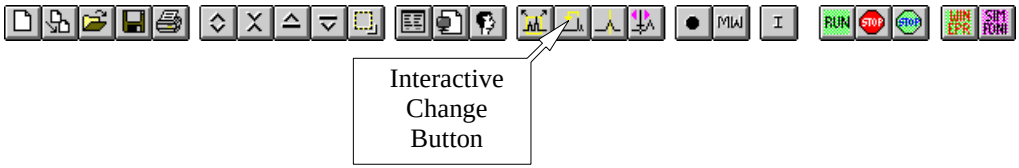


Figure 4-12 The Interactive Change of Center Field Parameter button in the tool bar.

Clicking this button creates a marker (vertical line) in the spectrum window that moves with the cursor. (See Figure 4-13.) Place the cursor where you would like the center field to be and click with the right mouse button. This action replaces the center field value with the magnetic field position of the marker.

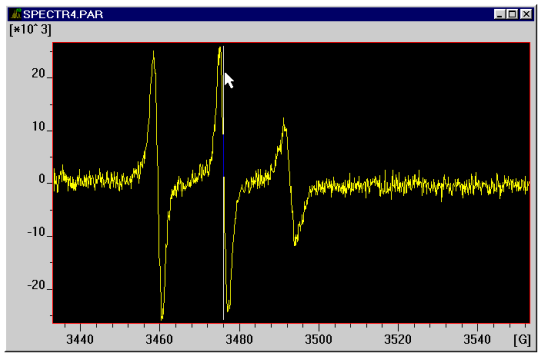


Figure 4-13 The center field marker.

To acquire the spectrum with the new center field, click on the RUN button in the tool bar. The newly acquired spectrum will then be nicely centered as in Figure 4-14.

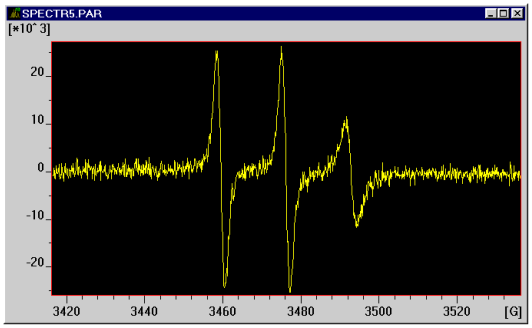


Figure 4-14 A centered spectrum.

Signal Averaging 4.3.3

Keep in mind, when measuring peak heights or double integration that you need to normalize the results by the number of scans.

If you are looking for very weak signals, you can increase your signal to noise ratio by signal averaging. This process involves repeatedly acquiring the spectrum and adding each spectrum together. Actually, this is not an average in the strict mathematical sense, (It is not normalized by the number of scans.) but is the sum of the individual spectra. As a result, the signal increases proportionally with N, the number of scans. Owing to the random nature of noise, its increase will only be proportional to $\sqrt{N}$. The resultant enhancement of signal to noise is then proportional to $\sqrt{N}$.

In order to signal average, we must open the Experiment Parameter dialog box. Enter the number of scans you wish to average. (See Figure 4-15.) In this example, 16 scans results in a four-fold improvement. (See Figure 4-16.) Acquire the spectrum by clicking the RUN button in the tool bar.

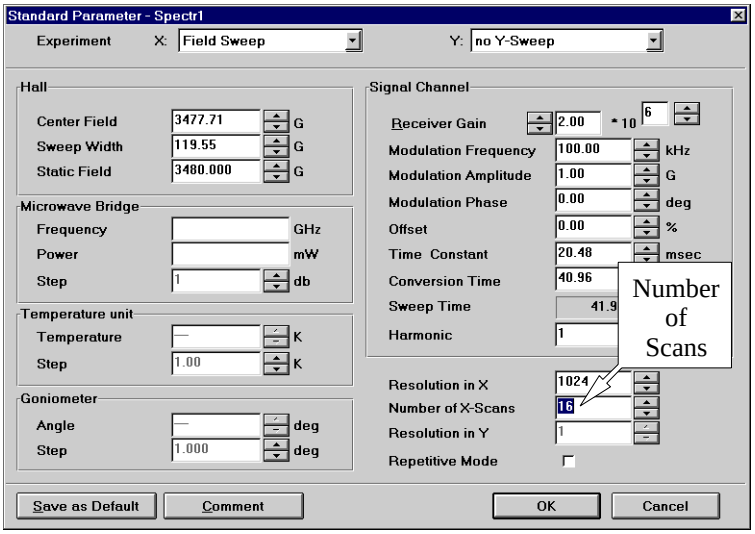
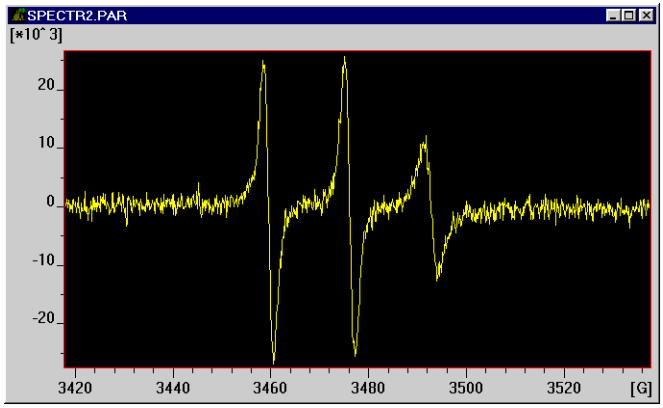
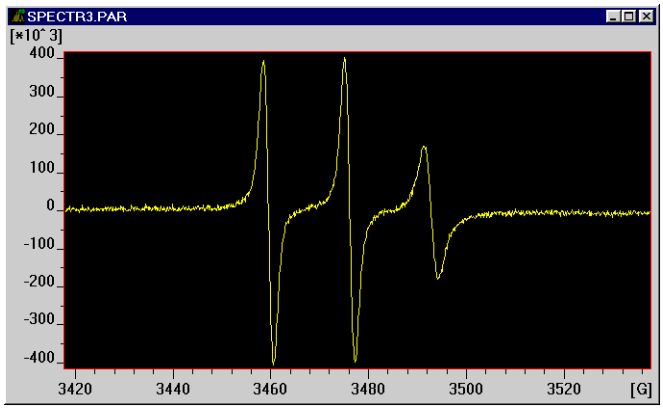


Figure 4-15 The Number of Scans parameter.



One Scan



Sixteen Scans

Figure 4-16 Improvement in signal to noise ratio through signal averaging.

Resolution 4.3.4

EPR spectra acquired with a computer consist of a list of magnetic field values and corresponding intensities. If you have very narrow lines, care must be taken that there are enough data points to fully characterize the lineshapes. If there is not sufficient resolution, expanded sections of the spectrum will only be crude approximations of the actual signal. The number of points in a spectrum can be chosen in the Experiment Parameter dialog box. (See Figure 4-17.) The parameter to adjust is the Resolution in X. (Alas, this is a misnomer, what is actually listed is the reciprocal of the resolution.) Perhaps the best way of demonstrating this effect is to look at the following two examples. The first spectrum was acquired with only 1024 points. (See Figure 4-18.) The second spectrum was acquired with 4096 points and reproduces the lineshapes much better than the first example. (See Figure 4-18.)

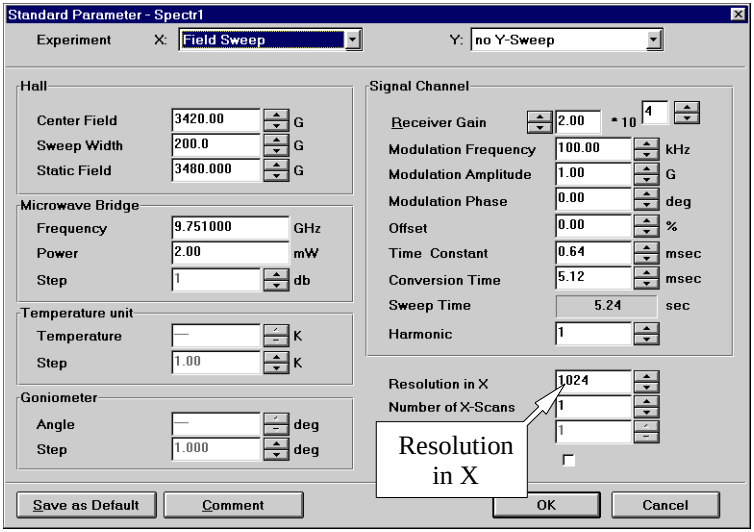


Figure 4-17 The Resolution in X parameter.

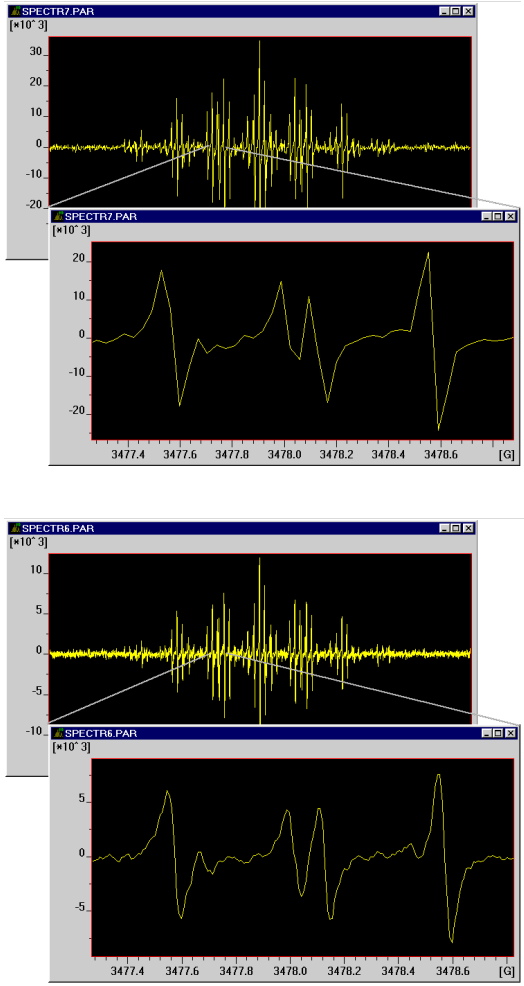


Figure 4-18 Perylene radical cation spectrum acquired with 1024 or 4096 points.

Time Scans 4.4

Not all radicals are very stable: in fact most are very reactive species and will disappear via chemical reactions. Many people are interested in the kinetics of these reactions. The EMX software has a Time Scan option to study the time behavior of such changing systems. The magnetic field is kept fixed at a specified value and the EPR signal intensity is monitored as a function of time.

The first task is to acquire a field swept spectrum before acquiring the time scan. This spectrum is usually acquired under steady-state conditions or before the chemical reactions have started.

Figure 4-19 Interactive Change of Static Field Parameter and Duplicate buttons in the tool bar.

In order to determine where to set our static field we must first duplicate the field swept spectrum by clicking the Duplicate button in the tool bar. Activate the new spectrum window and click the Interactive Change of Static Field Parameter button in the tool bar. (See Figure 4-19.) Clicking this button creates a marker (vertical line) in the spectrum window that moves with the cursor. (See Figure 4-20.) Place the cursor where you would like the static field to be and click with the right mouse button. This action replaces the default static field value with the magnetic field position of the marker.

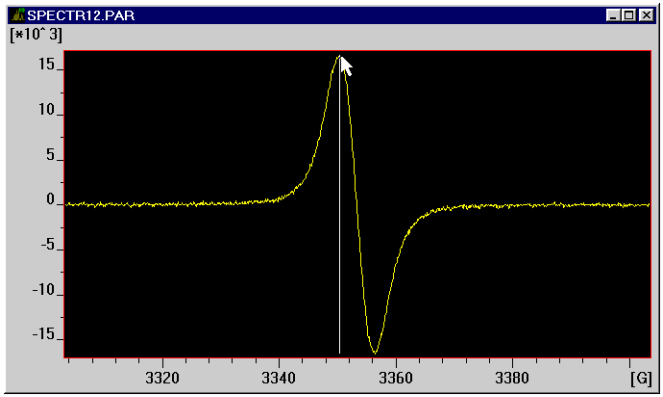


Figure 4-20 Interactive selection of the static field for a time scan.

Once the static field has been selected, we must switch from a Field Sweep to a Time Scan. Open the Experiment Parameter dialog box and select Time Scan. (See Figure 4-21.)

Change the other parameters such as the Conversion Time and Time Constant so that they are appropriate for the time scales to be encountered with the chemical reaction.

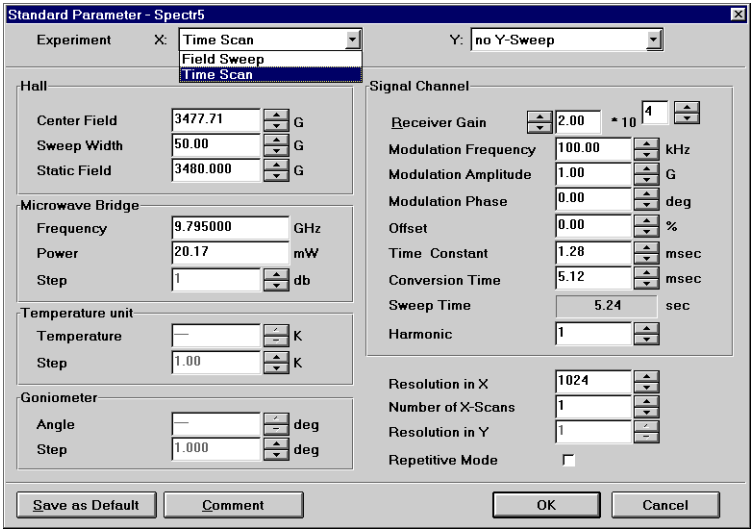


Figure 4-21 Selection of the Time Scan option.

There remains one more task before we can acquire the time scan: we must disable the Automatic Baseline Correction. The default option is to subtract the average value of the spectrum at the end of a scan. This feature is convenient for field sweep spectra because the average value should be zero if you sweep through the complete EPR spectrum. Subtraction of the average value therefore makes double integrations easier. A zero average value is not necessarily true for a time scan. You may also want to know the ratio of the initial and final intensity, which would be impossible if the average value were subtracted. To disable the Automatic Baseline Correction option, open the Experimental Options dialog box and click the box next to Automatic Baseline Correction so that the x disappears. (See Figure 4-22.)

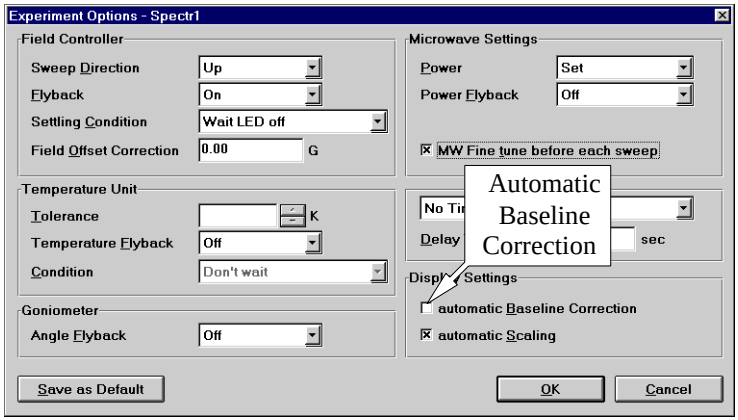


Figure 4-22 Selection of the Automatic Baseline Correction option.

Acquire the time scan by clicking the RUN button in the tool bar. (See Figure 4-23.) There may be a slight offset, particularly if your signal is very weak. Acquire another spectrum “off resonance” and subtract it from the first spectrum to get an accurate result.

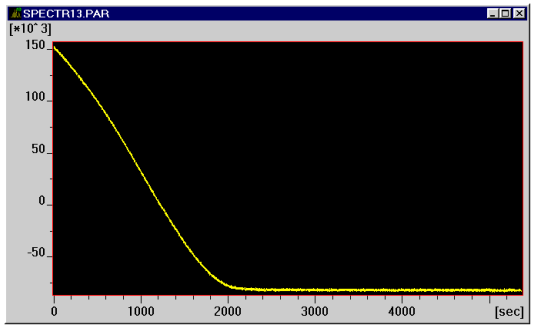


Figure 4-23 A time scan of a decaying radical.

Interactive Spectrometer Control 4.5

Immediate active visual feedback when parameters are changed helps you to optimize your spectrometer parameters. The Experiment Parameter dialog box does not change the parameters on the instrument until you run a spectrum, but the Interactive Spectrometer Control dialog box supplies you with the tools needed for interactive optimizations. Click the Interactive Spectrometer Control button in the tool bar (Figure 4-24) and the dialog box will appear. (See Figure 4-25.)

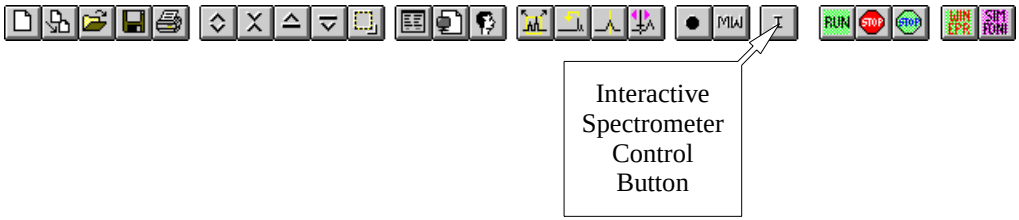


Figure 4-24 The Interactive Spectrometer Control button in the tool bar.

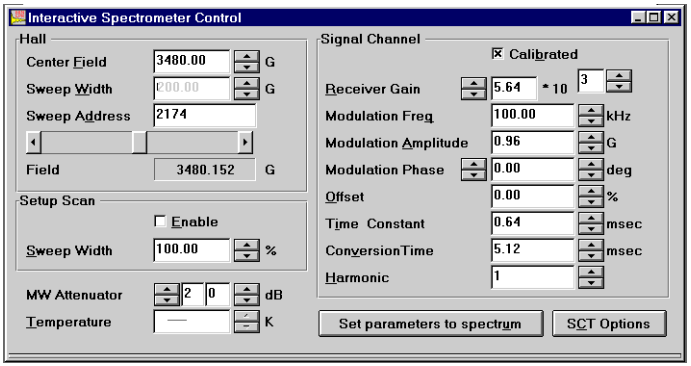


Figure 4-25 The Interactive Spectrometer Control dialog box.

It is best to move the Interactive Spectrometer Control dialog box up as high as possible before enabling the Setup Scan. The Setup Scan display will then be as large as possible.

To supply immediate feedback, the dialog box also has a Setup Scan option in which the magnetic field is rapidly swept up to 50 Gauss in order to display the EPR spectrum on the screen. This is achieved by setting the main magnetic field with the field controller (thereby setting the center field of the Setup Scan) and sending current through the modulation coils of the cavity to produce the rapid sweep. Click on the Enable button to activate the option. Use the field slider bar to center your spectrum in the setup scan. (See Figure 4-26.)

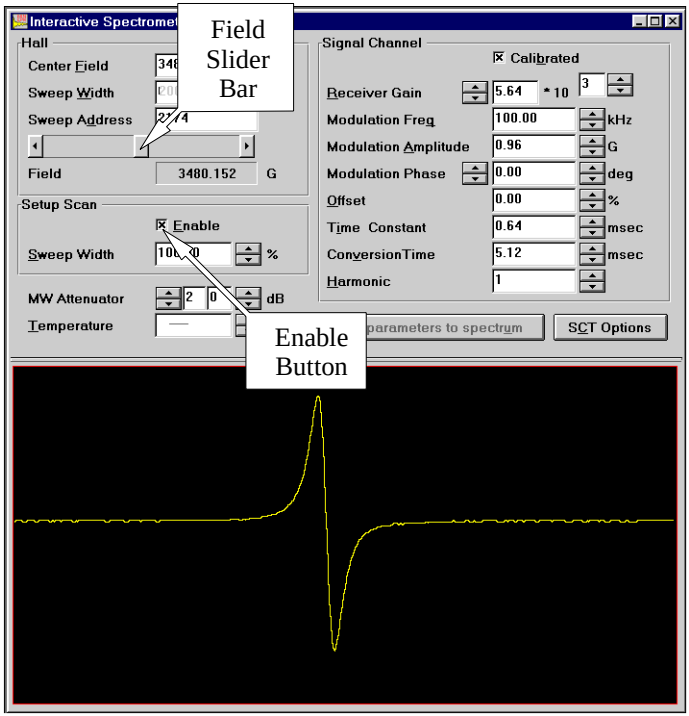


Figure 4-26 The Setup Scan.

The rapid sweep for the Setup Scan may be a little too fast for some signals or parameters. A time constant that does not distort

an EPR signal during a normal field sweep, is usually too long for the rapid field sweep of the Setup Scan and will distort the signal. (See Figure 4-27.) One means of dealing with this is to shorten the time constant. Another means is to narrow the width of the Setup Scan so that you are looking at a narrower portion of the signal. The time required to sweep through the spectrum is then longer. The number in the Sweep Width box indicated in Figure 4-27 can be edited or varied with the arrows next to it. The values are in percentage of the 50 G setup scan sweep.

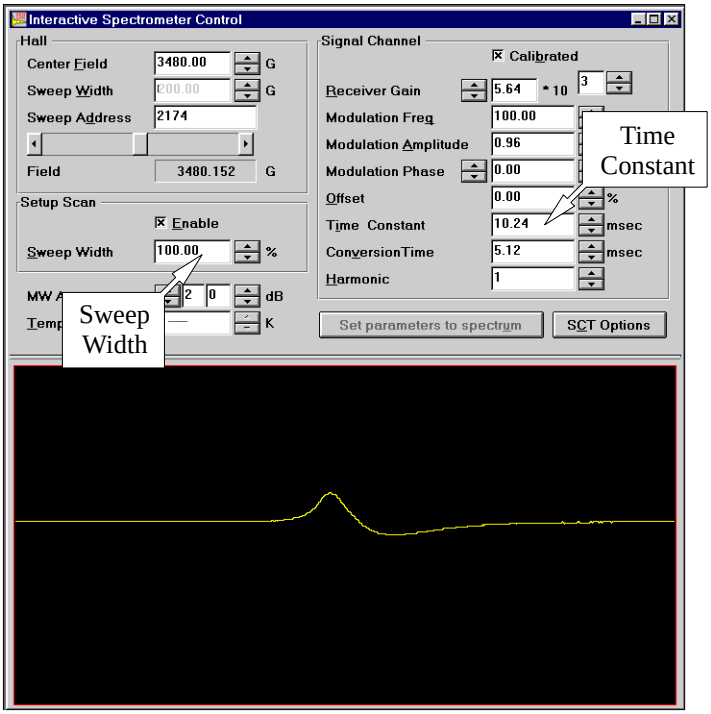


Figure 4-27 A distorted Setup Scan EPR spectrum.

If the EPR signal is very weak or very broad, the Setup Scan may not be the best way to optimize the signal. For such cases,

you can use the Interactive Receiver Level Display option in conjunction with the Interactive Spectrometer Control dialog box to optimize your EPR signals. First, make sure you have an acquired spectrum and that its window is active. Clicking the Interactive Receiver Level Display button in the tool bar (Figure 4-28) creates a marker (vertical line with a short horizontal bar) in the spectrum window. The position of this marker in the spectrum determines the magnetic field, *i.e.* changing its position changes the actual magnetic field. The short horizontal bar indicates the receiver level at the magnetic field at which the marker is placed. The marker is moved by placing the cursor at the desired position in your EPR spectrum and clicking the left mouse button. (Clicking the right mouse button makes the marker disappear.) Fix the cursor at the desired position in the spectrum and open the Interactive Spectrometer Control dialog box. Now, as you vary parameters such as receiver gain, phase, or microwave power, you can monitor the signal intensity at that magnetic field value. (See Figure 4-29.)

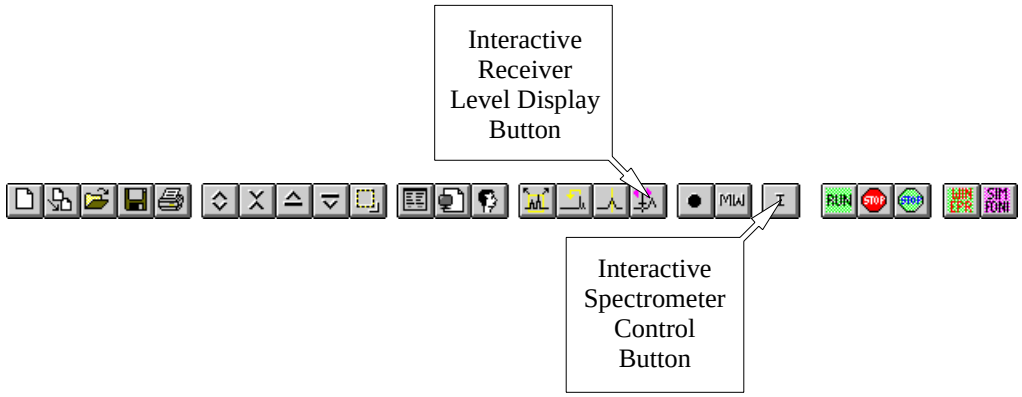


Figure 4-28 The Interactive Spectrometer Control and Interactive Receiver Level Display button in the tool bar.

The parameter values that you have carefully optimized have no effect on the spectrum that you have already acquired. To use these new parameters for a new acquisition, you need to set these values to a spectrum. To set these values to a spectrum, click on the Set parameters to spectrum button. The cursor will turn into the letter P (for Parameter). Place the cursor on the target spectrum window and click the left mouse button to copy the parameters to that spectrum. Reacquire a spectrum in that window, the spectrometer will use the newly optimized parameter set.

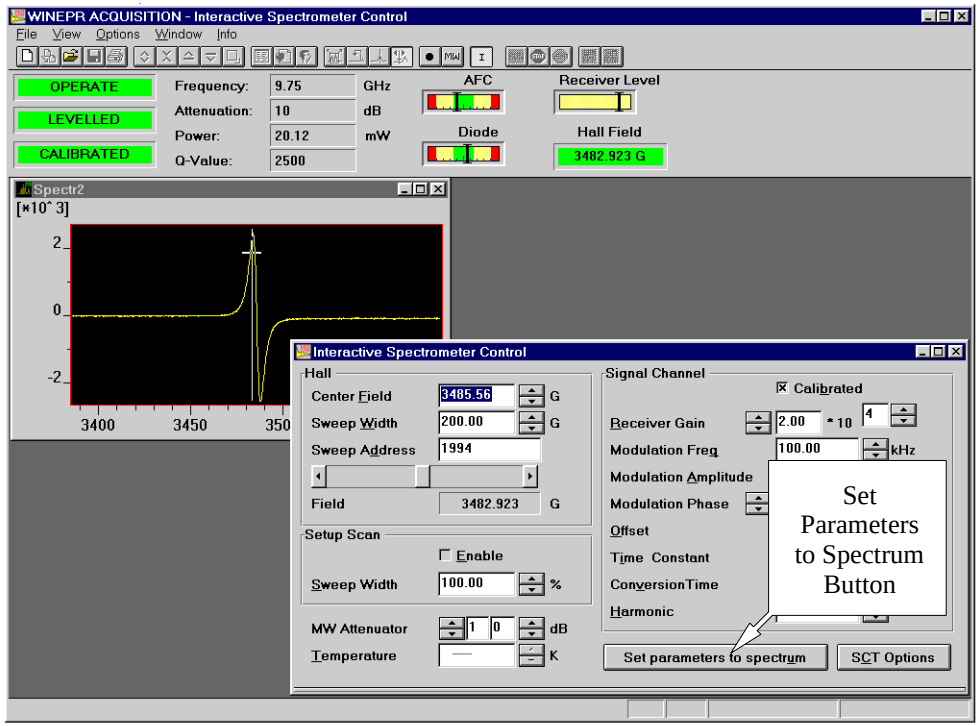


Figure 4-29 Use of the Interactive Receiver Level Display to optimize spectrometer parameters.

Controlling the Microwave Bridge 4.6

Auto Tune vs. Fine Tune 4.6.1

The time saved by using Fine Tune instead of the full Auto Tune procedure can be particularly important when you are working with unstable and decaying samples.

In Chapter 3, we used the Auto Tune commands to tune the microwave bridge and cavity. This routine tunes everything, including the Bias, Signal Phase, Frequency, and the cavity matching. Quite often, you do not need to adjust all these parameters. For example, unless you have a large change in microwave frequency the Bias and Signal Phase do not need to be adjusted. The parameters that change more frequently are the Frequency and the matching of the cavity. The Fine Tune routine optimizes only the Frequency and the matching (iris position) and therefore is considerably faster than the complete Auto Tune procedure.

A good approach to take is to initially use the Auto Tune routine to make sure that the Bias and Signal Phase are set properly. Then as you change samples (providing they have similar properties) or rotate your sample, *etc.*, you can use the Fine Tune routine to tune the spectrometer. You can either press the Fine Tune button in the tool bar (Figure 4-30) or open the Microwave Bridge Control dialog box and press the Fine Tune button (Figure 4-31).

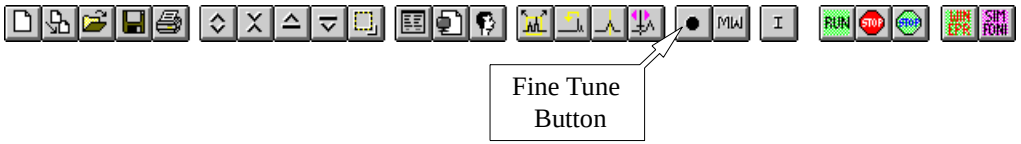


Figure 4-30 The Fine Tune button in the tool bar.

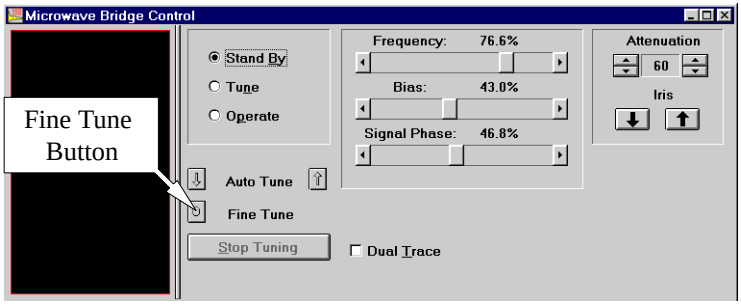


Figure 4-31 The Microwave Bridge Control dialog box.

You can set up an automatic fine tune before each sweep. Open the Experiment Options dialog box by pressing the button in the tool bar. Click the check box for MW Fine Tune Before Each Sweep. (See Figure 4-32.) Click OK and the fine tune routine will be executed automatically before each scan.

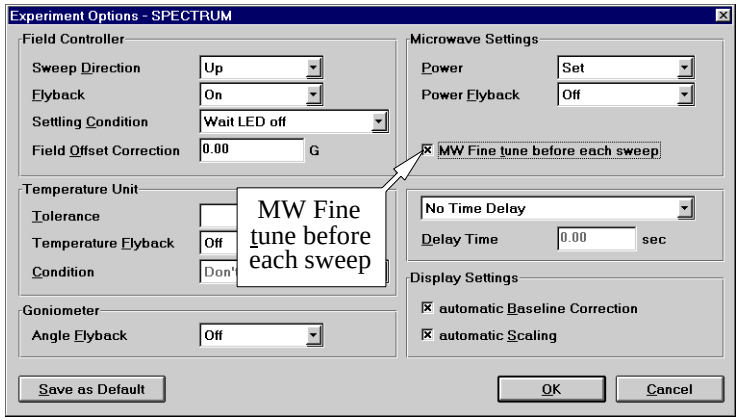


Figure 4-32 The MW Fine Tune Before Each Sweep check box.

Setting the Microwave Power 4.6.2

There are two options for setting the microwave power, Set and Read Only. Either option can be selected with the Microwave Settings drop-down list in the Experiment Options dialog box. (See Figure 4-33.)

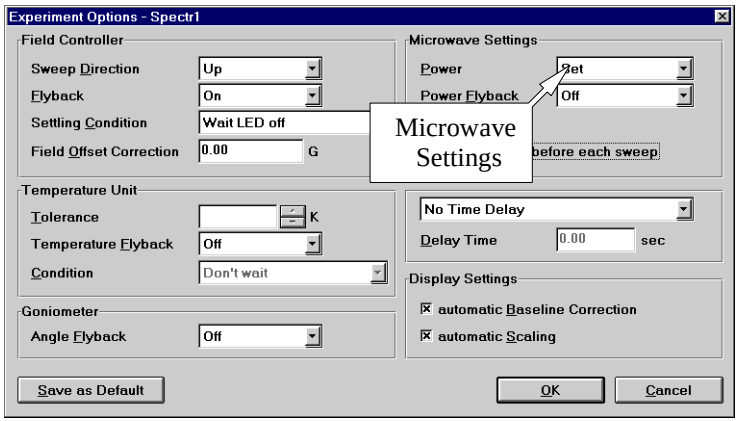


Figure 4-33 The Experiment Options dialog box.

In the Read Only mode, the microwave power is set by adjusting the attenuator in the Microwave Bridge Control or Interactive Spectrometer Control dialog box. The microwave power displayed in the Experiment Parameter box simply reflects the current microwave power. This mode is useful when you have interactively optimized the microwave power for an EPR experiment.

The opposite is true for the Set mode. In this mode, the microwave power is set to whatever value is displayed in or typed into the microwave power display. (See Figure 4-34.) The software determines the appropriate microwave attenuator value to obtain the specified microwave power. This mode is useful when you want to set the microwave power to a specific value.

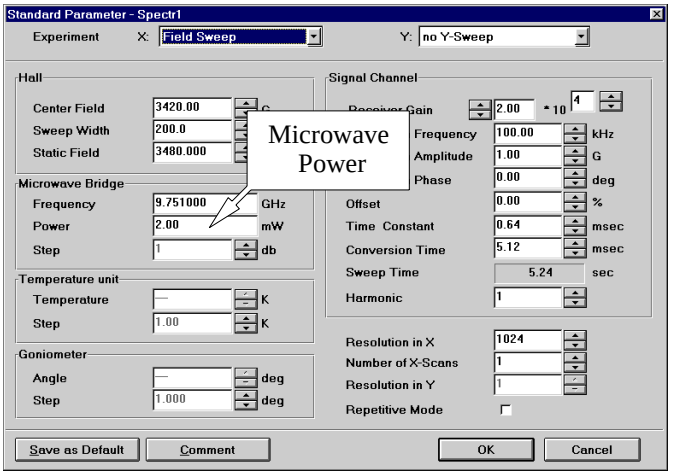


Figure 4-34 The Experiment Parameter dialog box.

Spectrum Files 4.7

Saving Files 4.7.1

The WIN-EPR Acquisition program stores all the spectra you see on the screen in memory. If you exit the application or turn your computer off, they are gone forever. You need to save your spectra to a disk for more permanent storage.

Saved spectra consist of two files. The first file is the spectrum file (*.spc file extension) that is a binary file containing all the intensities (y values) of the spectrum. The second file (*.par file extension) is a parameter file that is an ASCII file containing the parameter values used to acquire the spectrum. Whenever you save or open spectra, both the spectrum and parameter files are automatically saved or loaded.

After you have acquired a spectrum, you may save it in any folder. Click on the File menu bar and then click Save As. (An alternative would be to click on the Save button in the tool bar.) A dialog box appears that lets you choose a filename, a destination folder, and a destination disk drive. (See Figure 4-35.) The spectrum to be saved is the spectrum that is presently active. To select the appropriate disk drive, click on the arrow on the Drives: selector. To select the appropriate folder, click on the appropriate paths in the Folders: selector. The spectrum filename is selected by typing the filename in the File Name selector. Clicking OK saves the spectrum on the hard disk or diskette.

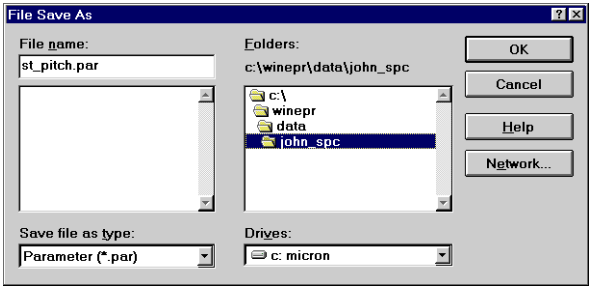


Figure 4-35 The File Save As dialog box.

If the chosen filename were already used by another file, a warning box gives you the opportunity to decide whether or not to replace the existing file with the present spectrum. (See Figure 4-36.) Pressing No cancels the save process and allows you to select another name or folder.

Figure 4-36 Warning dialog box for overwriting files.

Disk Housekeeping 4.7.2

The hard disk is the major storage device for storing acquired spectra. It can rapidly become cluttered and unwieldy. These type of problems are easily overcome if you implement an efficient folder system and if you back up your hard disc periodically.

Subdivide your folders when you are saving many spectra. Make sure that the folders have descriptive names so that it will be easier to find data in the future. For labs where many people use the instrument, it helps keep your data separate from those of others. Folders facilitate easy data transfer to floppy or to other computers via ethernet or Kermit. Creating folders is easy with either My Computer or Windows Explorer. Consult your Microsoft Windows™ documentation for further details.

Sending Spectra for Processing 4.8

Sending Spectra to WIN-EPR and *SimFonia* 4.8.1

You can directly send a spectrum to WIN-EPR for immediate processing. Click the spectrum you want to process to activate the spectrum window and then click the Send Spectrum to WIN-EPR button in the tool bar. (See Figure 4-37.) The WIN-EPR data processing program will open with the spectrum automatically loaded. Consult the WIN-EPR documentation about details of data processing.

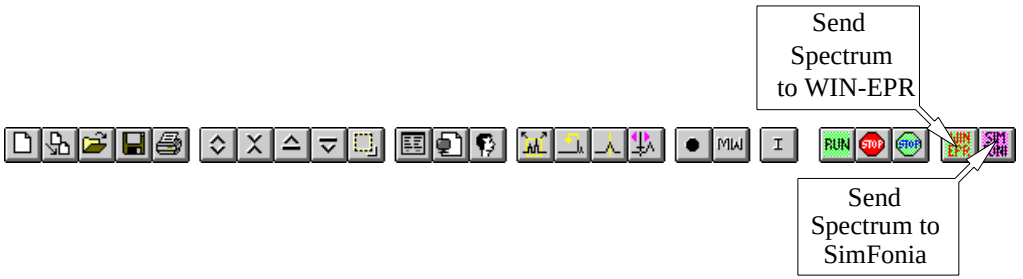


Figure 4-37 The Send Spectrum to WIN-EPR button and Send Spectrum to SimFonia button in the tool bar.

You can also send a spectrum to the *SimFonia* program for simulation without quitting the Acquisit program. Click the spectrum you want to simulate to activate that spectrum window and then click the Send Spectrum to SimFonia button in the tool bar. (See Figure 4-37.) The *SimFonia* program will open with the spectrum automatically loaded. Consult the *SimFonia* documentation for details about simulations.

Additional Techniques 5

This chapter provides instructions for procedures that are routine for some users, but may be infrequently encountered by others. Specifically, the chapter will describe manually tuning the EMX spectrometer, changing cavities, fine tuning the AFC, and performing automated 2D experiments.

Manually Tuning a Microwave Bridge 5.1

The Auto Tune routine of the EMX software is effective at tuning the cavity and bridge under most circumstances. However, there are some circumstances where automatic tuning may have difficulties. Lossy samples such as water can be problematic, particularly when you work at high microwave power levels. Following these instructions will help you to tune the spectrometer under these adverse conditions.

1. **Open the Microwave Bridge Control dialog box.** If this window is not already open, click its button (the button labeled MW) in the tool bar. The button toggles the dialog box open and closed. The microwave bridge control dialog box will then appear. (See Figure 5-1.)

A klystron bridge requires approximately three minutes to warm up after the console is turned on. When the Stand By indicator is green, the software allows you to switch to Tune mode.

2. **Switch the microwave bridge to Tune mode.** The bridge status indicator shows the three states or modes for the microwave bridge, Stand By, Tune, and Operate. (See Figure 5-1.) In Stand By the power to the microwave source is shut off. When you switch to Tune, the source turns on and you produce a frequency sweep that allows you to see the dip of your cavity. Switching to Operate causes power only at the resonant frequency to be transmitted to the cavity. When you turn on your spectrometer, it should be in Stand By mode, which is indicated by Stand By appearing in the Microwave Bridge Control menu. (See Figure 5-1.) If you have been acquir-

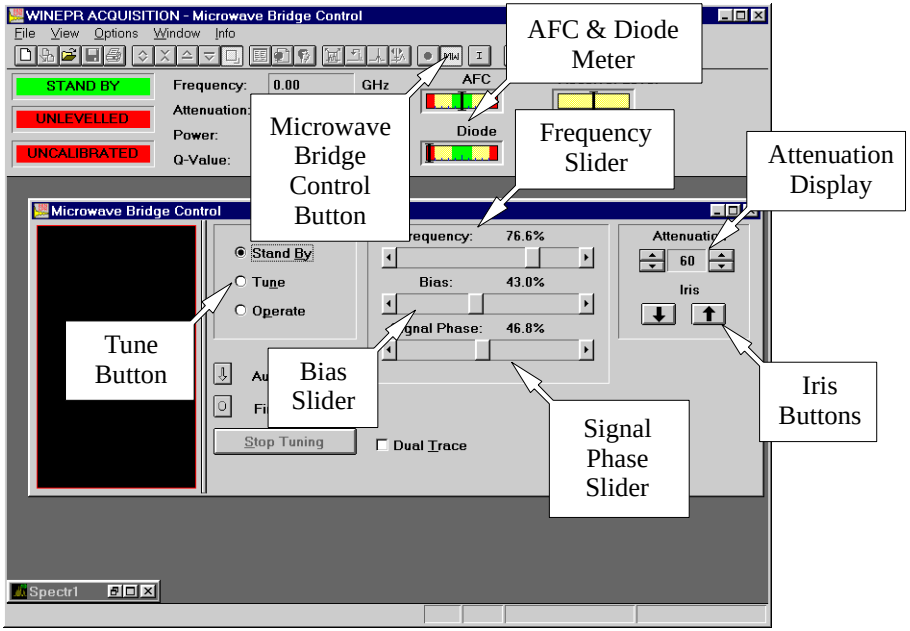


Figure 5-1 The Microwave Bridge Control dialog box.

You may notice that LEVELED and UNCALIBRATED appear in the bridge status indicator. Do not be alarmed by the UNCALIBRATED indicator; this is normal during Tune.

ing spectra already, your bridge will probably be in Operate mode. Click the Tune button in the dialog box to change to the Tune mode.

3. **Set the microwave attenuator to 25 dB.** The microwave attenuation is set by clicking the arrows on either side of the attenuation display. (See Figure 5-1.) The arrows on the left side change the attenuation in 10 dB steps; the arrows on the right side change the attenuation in 1 dB steps.

There are two types of microwave sources. The letter G in the microwave bridge designation (i.e ER 041 XG) on the front panel identifies a Gunn source. The letter K designates a klystron source. Perhaps the surest method to identify the type of source is by comparing the mode pattern with either Figure 5-2 or Figure 5-3.

4. **Observe the mode pattern on the display monitor. (Gunn Diode Microwave Sources)** This mode pattern is a display of the microwave power reflected from the microwave cavity and the reference arm power as a function of the microwave frequency. The mode pattern should resemble one of the mode tuning patterns in Figure 5-2. If the mode pattern amplitude is too small, increase the microwave power in 1 dB steps by decreasing the attenuation. If the mode pattern amplitude is too large, decrease the microwave power in 1 dB steps by increasing the attenuation.

5. **Observe the mode pattern on the display monitor. (Klystron Microwave Sources)** This mode pattern is a display of the microwave power reflected from the microwave cavity and the reference arm power as a function of the microwave frequency. The mode pattern should resemble one of the mode tuning patterns in Figure 5-3. If the mode pattern amplitude is too small, increase the microwave power in 1 dB steps by decreasing the attenuation. If the mode pattern amplitude is too large, decrease the microwave power in 1 dB steps by increasing the attenuation.

Figure 5-2

Mode tuning patterns for a Gunn diode microwave source.

a) Off resonance.

b) Slightly off resonance

c) On resonance, phase 180° off.

d) On resonance, phase 90° off.

e) On resonance, correct phase, undercoupled.

f) On resonance, correct phase, overcoupled.

g) On resonance, correct phase, critically coupled.

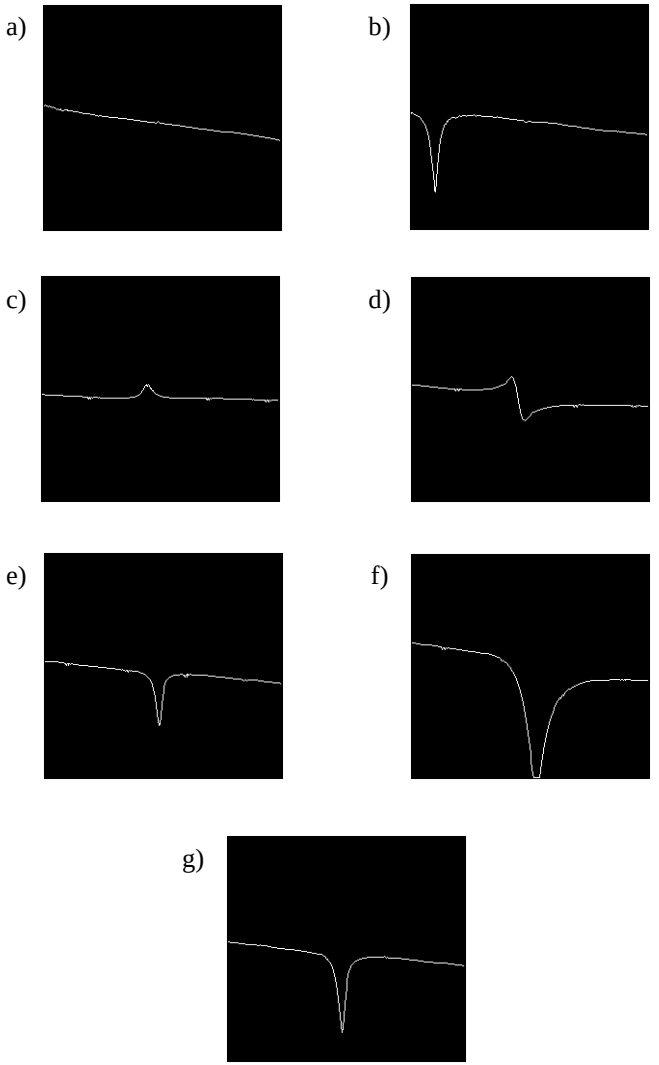
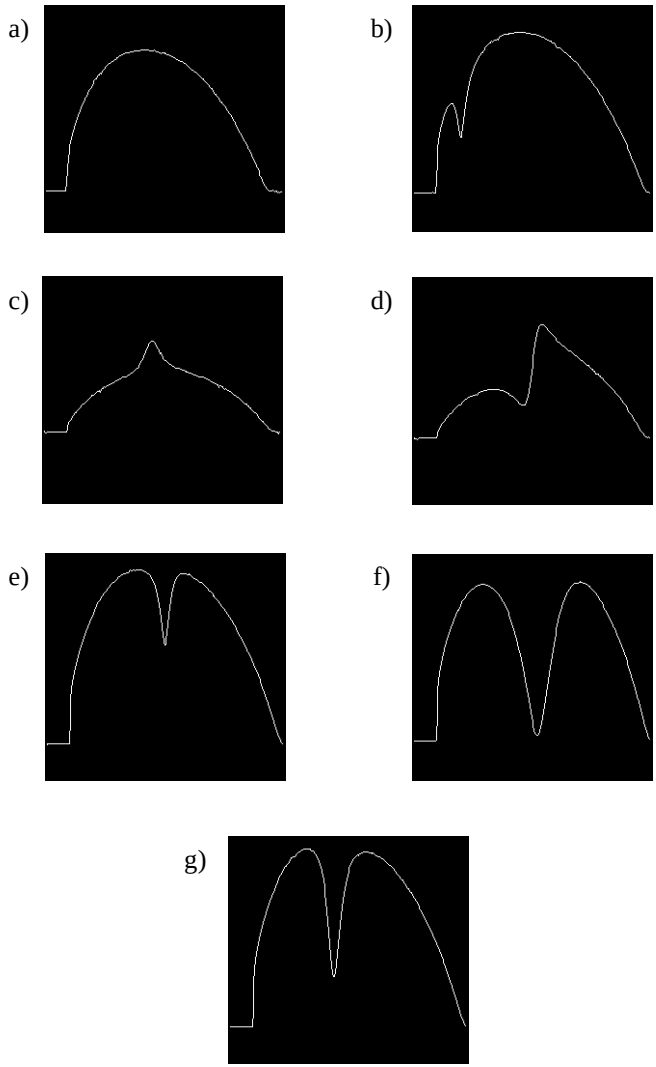


Figure 5-3



Mode tuning patterns for a klystron microwave source.

a) Off resonance.

b) Slightly off resonance

c) On resonance, phase 180° off.

d) On resonance, phase 90° off.

e) On resonance, correct phase, undercoupled.

f) On resonance, correct phase, overcoupled.

g) On resonance, correct phase, critically coupled.

The resonant frequency of a Bruker ER 4102ST cavity is usually approximately 9.8 GHz.

A cryostat will drop the frequency to approximately 9.4 GHz.

6. **Tune the microwave source.** Adjust the Frequency slider bar to locate and center the mode pattern “dip” on the display monitor. Clicking the left or right arrows will step the parameter value downwards or upwards. Clicking to the left or right of the square steps the parameter value downward or upward faster than when using the arrows. Keeping the mouse button pressed repeats the action automatically. The value of the parameter is indicated graphically by the position of the square in the slider bar. You can also vary the parameter by clicking and dragging the square. The “dip” corresponds to the microwave power absorbed by the cavity, and thus, is not reflected back to the detector diode. By centering the “dip” on the display monitor, the microwave source is set to oscillate at the same frequency as the cavity resonant frequency.

7. **Clean the sample tube to be inserted into the cavity.** Wiping the outside of the sample tube with tissue paper is usually adequate. It is vital to avoid contaminating the microwave cavity as paramagnetic contaminants may result in spurious EPR signals or distorted base lines in your EPR spectra.

8. **Insert the sample tube carefully into the cavity.** (See Figure 5-4.) Make sure you have the appropriate collet size for your sample tube size. The tube should be slightly loose before you tighten the collet nut. The bottom of your sample should rest in the indentation on the pedestal. This ensures that your sample is centered horizontally. If you have a small sample (less than 2 cm in length), you should visually judge how far the tube should go into the cavity in order to vertically center the sample in the cavity. You can adjust the sample position by loosening the bottom collet nut and moving the pedestal up and down. Make sure that the pedestal is not in the cavity, as it can give an EPR signal. Tighten the top collet nut to firmly hold the sample tube in place and the bottom collet to firmly hold the pedestal.

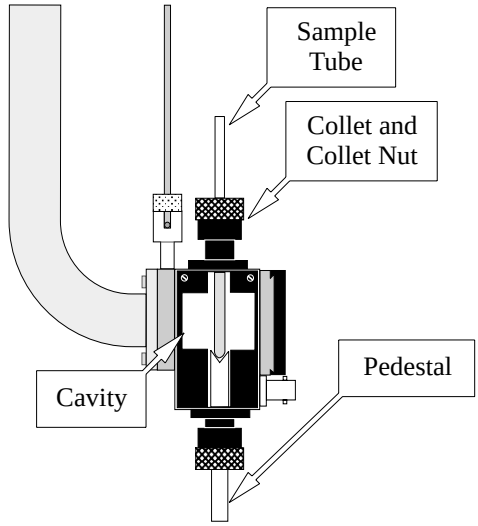


Figure 5-4 Cutaway view of a Bruker ER 4102ST cavity.

9. **Retune the microwave source.** Repeat the procedure of Step 6. You may notice a shift in the frequency, width, and depth of the cavity “dip” when you insert the sample. This is an indication that the microwave field patterns in the cavity are perturbed by the sample and tube. Lossy and conductive samples will appreciably perturb the field patterns, resulting in large shifts in the resonant frequency. Highly conductive samples tend to increase the resonant frequency by decreasing the effective cavity volume. Lossy samples will decrease the resonant frequency because of their large dielectric constants.

Steps 10 and 11 assume you have bias in the reference arm. (You usually do!) If the Bias slider bar (See Figure 5-1.) is all the way to the left side, move it towards the middle to ensure sufficient bias. Otherwise, leave it be.

10. **Tune the signal (reference) phase. (Gunn Diode Microwave Sources)** While the “dip” is in the center of the display, adjust the Signal Phase slider bar (See Figure 5-1.), until the depth of the dip is maximized and the “dip” looks somewhat symmetric. (See Figure 5-2.) We shall fine-tune this phase later, but this procedure gets us close to the correct phase.

11. **Tune the signal (reference) phase. (Klystron Microwave Sources)** While the “dip” is in the center of the display, adjust the Signal Phase slider bar (See Figure 5-1.), until the shoulders on each side of the “dip” appear to be approximately the same height and the “dip” looks somewhat symmetric. (See Figure 5-3.) We shall fine-tune this phase later, but this procedure gets us close to the correct phase.

12. **Fine-tune the microwave source frequency.** Click the Operate button in the dialog box to change to the Operate mode. Adjust the Frequency slider bar until the needle of the AFC meter is centered. You can locate the AFC meter by referring to Figure 5-1. Sometimes the needle may rush off to the right or left edges of the meter. This happens when the AFC (Automatic Frequency Control) is no longer locked. If this happens, click the Tune button to return to the Tune mode. Repeat Step 9. and then try again.

13. **Adjust the bias level.** Change the microwave attenuation to 50 dB. Adjust the Bias slider bar (See Figure 5-1.), until the Diode meter needle is centered. You can locate the Diode meter by referring to Figure 5-1. The center corresponds to 200 microamperes of diode current. Sometimes, particularly when the cavity has a low Q, the AFC meter may rush off either to the right or left and lose lock at 50 dB. In most cases, the AFC will lock again at higher microwave power levels. If not, switching between Operate and Tune modes and back again at 30 dB attenuation will lock the AFC once more.

14. **Match the cavity.** For maximum sensitivity, we need to critically couple (or match) the cavity to the waveguide. Critical coupling results in a maximum power transfer between the waveguide and the cavity. It also means that no incident microwaves are reflected back from the cavity. If the cavity and waveguide are truly matched, the reflected microwave power seen by the detector should remain constant (i.e. 0) when we vary the attenuation. This is the criterion we use for critical coupling. You control the coupling or matching of the cavity by adjusting the iris screw. First, increase the microwave power by 10 dB. (i.e. attenuator setting 40 dB). Click the ↑ or ↓ iris buttons for the iris screw motor until the diode current again returns to 200 microamperes. (i.e. The needle is

centered.) Repeat the procedure (-10 dB steps in the attenuator setting and adjust the current to 200 microamperes with the iris screw) until you have reached an attenuator setting of 10 dB. You will notice that as you increase the microwave power, the diode current becomes more sensitive to the position of the iris screw. Another thing you may notice is that the AFC meter also changes with the iris screw position. Simply adjust the frequency slider bar until the needle is centered again. When you have reached 10 dB microwave attenuation, adjust the Signal Phase slider bar until you achieve a local maximum in the diode current. You should not have to adjust it very much. Verify that you have achieved critical coupling by changing the microwave attenuation from 10 dB to 50 dB with virtually no change in the diode current. Repeat the matching and bias level adjustment procedures if necessary. If you need to operate at power levels greater than 20 mW (10 dB), set the attenuator to 0 dB and once again adjust the diode current to 200 microamperes with the iris screw. The current can sometimes drift because the high microwave power starts to heat the sample. If this happens, wait a minute or two and readjust the coupling.

Changing EPR Cavities 5.2

1. **Open the Interactive Spectrometer Control dialog box.** If this window is not already open, click its button (See Figure 5-5.) in the tool bar. The button toggles the dialog box open and closed. The Interactive Spectrometer Control dialog box will then appear. (See Figure 5-6.)

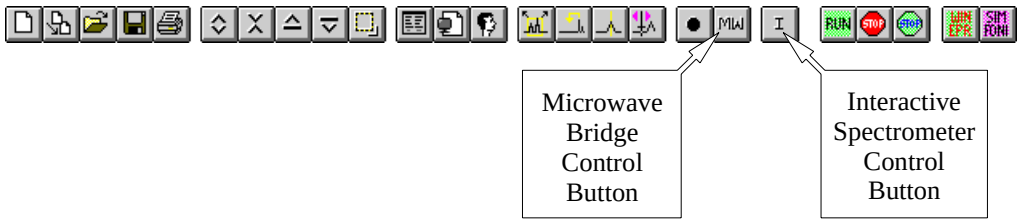


Figure 5-5 The Interactive Spectrometer Control button.

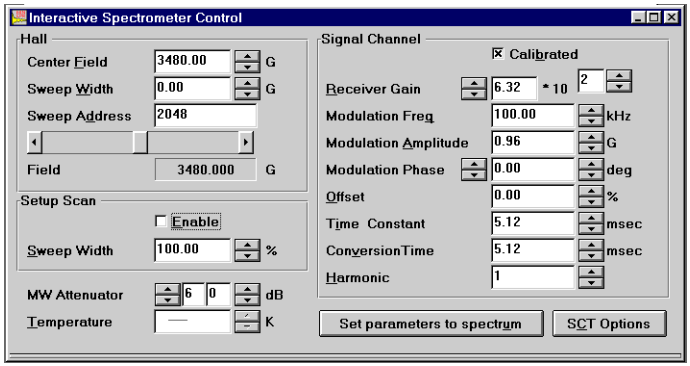


Figure 5-6 The Interactive Spectrometer Control dialog box.

Setting the magnetic field to the minimum value avoids the risk of magnetizing your watch when changing cavities.

2. **Set the modulation amplitude to zero.** Enter a value of 0.00 in the Modulation Amplitude box.

3. **Set the magnetic field to the minimal value.** Enter in a value of 0.00 in the Sweep Width box and a value of 0.00 in the Center Field box.

4. **Close the Interactive Spectrometer Control dialog box.** Click the Interactive Spectrometer Control (the button labeled **I**) in the tool bar. The button toggles the dialog box on and off. The Interactive Spectrometer Control dialog box will then disappear. (See Figure 5-5 and Figure 5-6.)

5. **Open the Microwave Bridge Control dialog box.** If this window is not already open, click its button (See Figure 5-5.) in the tool bar. The button toggles the dialog box open and closed. The Microwave Bridge Control dialog box will then appear. (See Figure 5-7.)

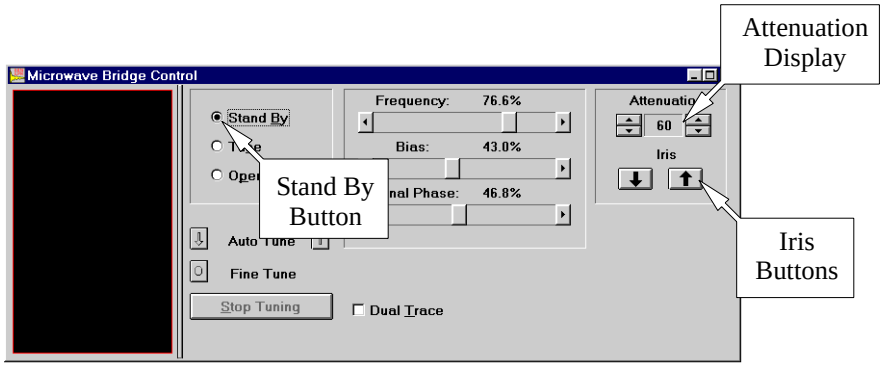


Figure 5-7 The Microwave Bridge Control dialog box.

6. **Switch the microwave bridge to Stand By mode.** Click the Stand By button in the dialog box to change to the Stand By mode. (See Figure 5-7.)

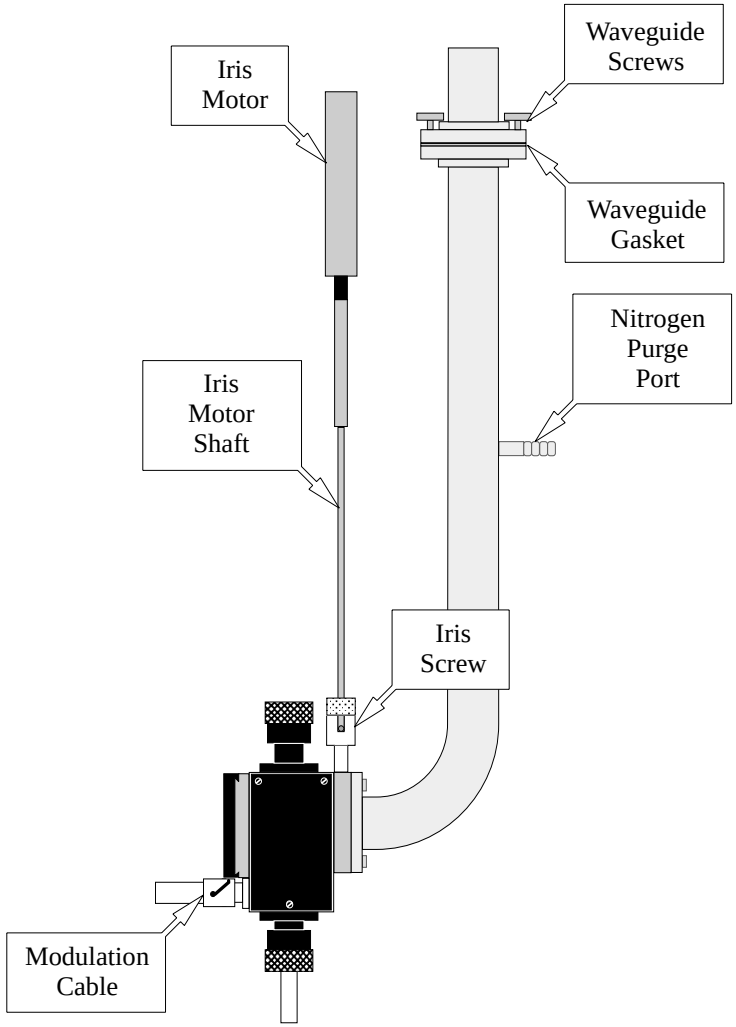


Figure 5-8 Connections on the ER 4102ST cavity.

7. **Disconnect accessories.** If a variable temperature dewar assembly is installed, disconnect the coolant transfer line and the thermocouple connections from the cavity.

8. **Disconnect the modulation cable from the cavity.** This is the twinax cable labeled with a white connector and attached to the front of the cavity. (See Figure 5-8.)

9. **Disconnect the nitrogen purge line from the port on the waveguide.** The port is half way down the waveguide attached to the cavity. (See Figure 5-8.)

Store the lock nut in a place where it will not be lost.

10. **Disconnect the iris motor shaft from the iris screw.** First unscrew the lock nut from the iris screw. Lift the shaft upwards to disconnect. Move the iris motor to the side where it is out of the way. (See Figure 5-9.)

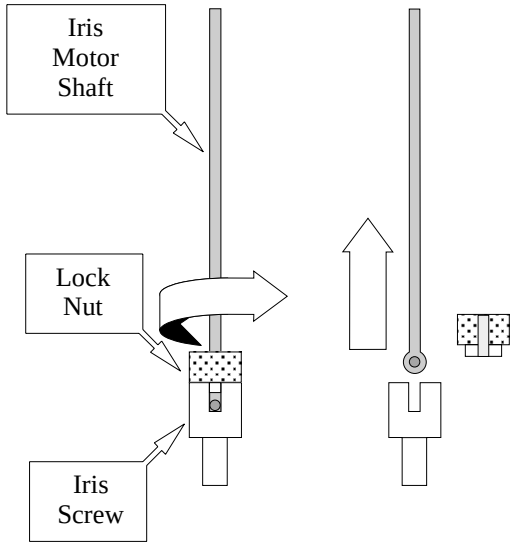


Figure 5-9 Disconnecting the iris motor shaft from the iris screw.

11. **Disconnect the cavity.** (See Figure 5-8.) While grasping the waveguide attached to the cavity with one hand, unscrew the four waveguide screws joining the two sections of waveguide. Loosen the waveguide stabilizers rotating the screws and carefully remove the cavity from the air gap of the magnet. (See Figure 5-10.) Take care not to lose the gasket which was between the two wave guide flanges. Seal the cavity with the solid collets and put the cavity in a safe clean place.

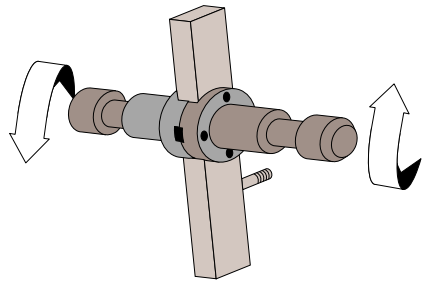


Figure 5-10 Loosening the waveguide stabilizers.

12. **Install the waveguide stabilizers on the new cavity.** (See Figure 5-11.) Visually position them just above the magnet pole caps.

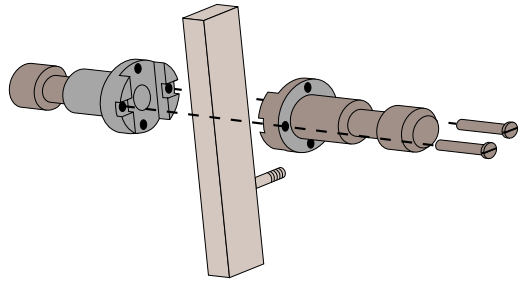


Figure 5-11 Installing the waveguide stabilizers.

Steps 14. and 15. are used to set the limit switches in the iris motor. The limit switches prevent you from screwing the iris in too far and thereby breaking the iris screw.

13. **Attach the appropriate size collet and pedestal on the cavity.**

14. **Screw in the iris. Manually turn the iris screw until it is almost all the way in.** The iris screw will stop rotating. It may be a good idea to back the screw out 1/2 turn after it hits the bottom. This will further decrease your chances of accidentally breaking the iris screw during the tune procedure.

15. **Click and hold the down Iris Button.** Activate this button (See Figure 5-1.) until the iris motor stops; this is the lower limit of the motor. With the iris motor in its lower limit, reattach the iris motor drive to the iris screw.

16. **Connect the modulation cable to the cavity.**

Make sure you connect the modulation cable to the MOD (modulation) connector and not the R.S. (Rapid Scan) connector.

17. **Reconnect the waveguide sections and tighten the stabilizers.** Do not forget to install the waveguide flange gasket between the two flanges; make sure it is oriented correctly. (See Figure 5-12.) Position the cavity in the center of the magnet air gap by moving the bridge on the table. Carefully tighten the stabilizers. Be careful not to stress the waveguide when expanding the stabilizers. Reconnect the nitrogen purge line and adjust the flow rate for a light flow.

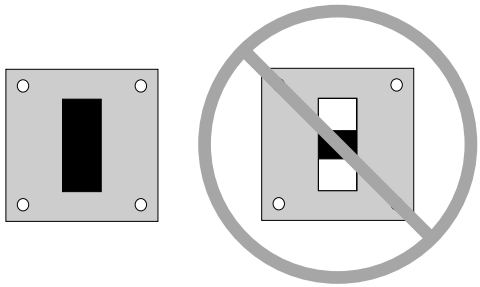


Figure 5-12 Installing the waveguide gasket properly.

18. **Reconnect the iris motor shaft to the iris screw.** The procedure here is like Step 10. performed in reverse. Reposition the iris screw motor. Screw the lock nut onto the iris screw. Click and hold the up iris button in the Microwave Bridge Control dialog box until the iris screw is approximately half way out.

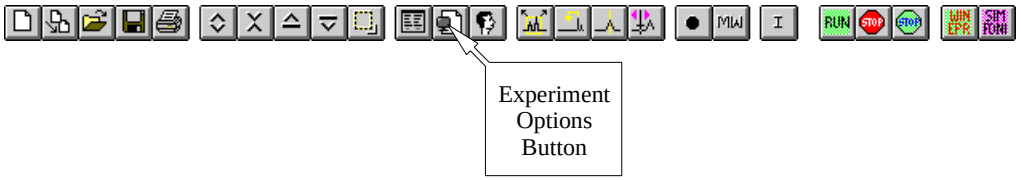


Figure 5-13 The Experiment Options button.

19. **Read in the calibration file for the cavity.** Open the Experiment Options dialog box in order to read in the calibration information. If this window is not already open, click its button (See Figure 5-13.) in the tool bar. The button toggles the dialog box open and closed. The Experiment Options dialog box will then appear. Click on the Change File button. A new dialog box, Open Calibration File will appear. Select the appropriate calibration file for your cavity and click OK. This will automatically load the calibration data you have selected. Confirm that the calibration file is the correct one for the cavity. The calibration file name usually consists of two or three letters that identify the type of cavity (ST for ER 4102ST or TM for ER 4103TM) followed by the serial number of the cavity. This number is located on either the front or back of the cavity. Clicking Cancel returns you to the Experiment Options dialog box.

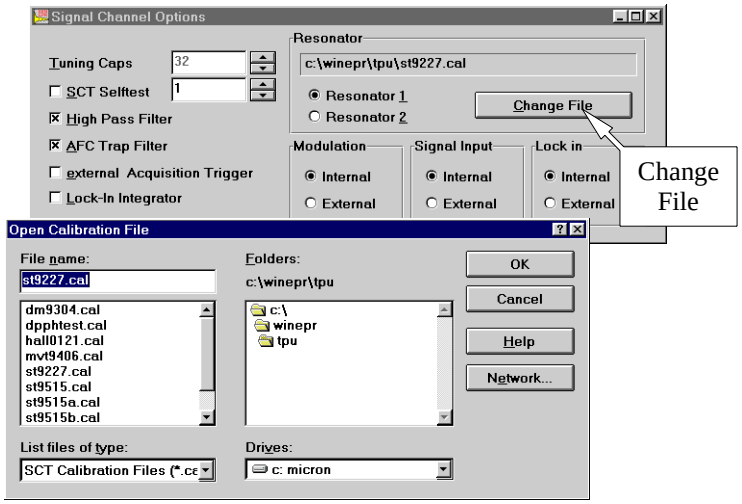


Figure 5-14 The Experiment Options and Open Calibration File dialog boxes.

Service engineers often save the calibration files in the c:\...\acquisit\tpu directory during the installation of the spectrometer.

Fine AFC Tuning for Gunn Diode Bridges 5.3

The AFC (Automatic Frequency Control) is the circuitry used to "lock" the microwave source frequency to the resonant frequency of the cavity. In most cases, particularly if the microwave attenuation is less than 40 dB, the AFC works very well without any need for you to fine-tune it. If you are performing experiments in which low microwave powers are required, following the instructions in this section will ensure that you will obtain optimal AFC performance. Please note that this procedure is not required for klystron bridges. You can determine the type of bridge you have by looking at the model designation on the front plate of the bridge. A model designation containing a G, for example ER 041 XG, indicates a microwave bridge with a Gunn diode microwave source. In contrast, a bridge with a model designation with a K, such as ER 041 XK, has a klystron microwave source.

The Fine-tuning Procedure 5.3.1

1. **Set the FINE AFC potentiometer to zero.** The potentiometer for the AFC can appear in two different locations on the bridge depending on when your bridge was manufactured. (See Figure 5-15.)

2. **Tune the microwave bridge.** Follow the procedures in Section 3.4 for automatic tuning or Section 5.1 for manual tuning. The frequency, bias, phase, and iris screw should be adjusted so that the needles of the AFC and Diode meters remain centered as you change the microwave attenuation from 0 to 40 dB. (See Figure 5-16 and Figure 5-17.) Note that there may be a drift at 0 dB

caused by sample heating if you have a lossy sample in the cavity.

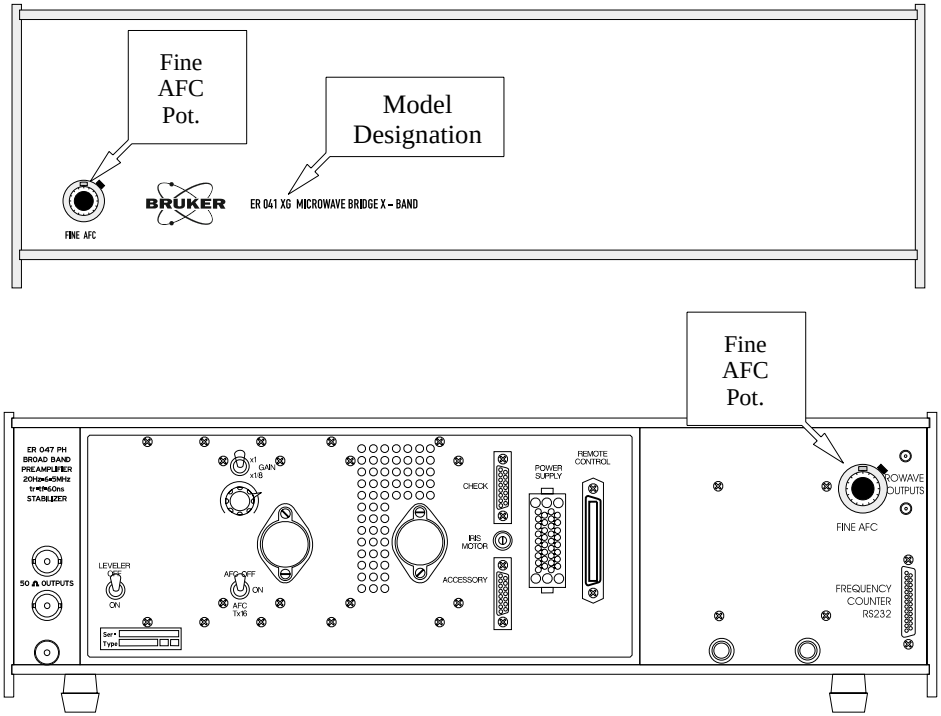


Figure 5-15 Two possible locations for the fine AFC potentiometer.

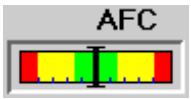


Figure 5-16 Properly centered AFC meter.

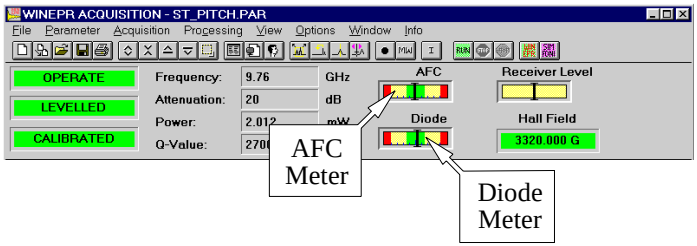


Figure 5-17 Location of the AFC and diode meters.

3. **Switch the microwave attenuation from 40 dB to 50 dB.** The AFC meter may drift to the right. (See Figure 5-18.)

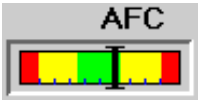


Figure 5-18 The AFC needle drifting towards the right.

4. **Increase the microwave attenuation slowly.** Increase the attenuation in 1 dB increments between 50 and 60 dB until you observe a significant deflection of the needle. (See Figure 5-19.)

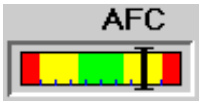
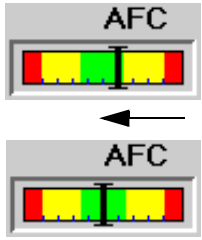


Figure 5-19 A significant AFC needle deflection.

5. **Adjust the FINE AFC Potentiometer.** Turn the knob until the AFC needle is once again centered in the AFC meter (Figure 5-20.).



Adjust the needle to the center

Figure 5-20 Centering the AFC meter.

6. **Verify that the AFC needle remains centered.** Vary the microwave attenuation between 0 and 60 dB. Note that there may be a drift at 0 dB caused by sample heating if you have a lossy sample in the cavity. Also, the needle may rush off to the left or right at low powers because the AFC loses lock. In most cases, the AFC will lock again at higher microwave power levels. If not, switching between Operate and Tune modes and back again at 30 dB attenuation will lock the AFC once more. Then increase the attenuation more slowly than the previous time. Repeat Step 2. through Step 6. until the needle remains centered.

7. **Record the microwave frequency and FINE AFC potentiometer setting.** The setting is microwave frequency dependent and reproducible. If you record the setting at that microwave frequency, you need not perform this whole procedure every time you use low microwave power levels. Because only the insertion of a cryostat substantially shifts the microwave frequency, you will typically only need a setting for a cavity with and without a cryostat.

Performing 2D Experiments 5.4

Using the WIN Acquisition software you can perform experiments in which a second parameter (*i.e.*, in addition to the magnetic field) can be varied. For example, you can perform a set of experiments in which the power is increased incrementally over several successive field scans. Alternatively, you might perform several consecutive experiments in which the temperature is ramped either up or down between each field scan. You can then display the 2D dataset using WIN-EPR. This section will describe how to utilize the Acquisition software to create a 2D data set and how to display it in WIN-EPR. The procedure is more easily described by performing an example experiment that investigates the response of the strong pitch spectrum to microwave power.

1. **Insert the strong pitch sample.** Place the strong pitch sample into the cavity and tune the spectrometer as described in either Section 3.4 or Section 5.1.

2. **Open the Experimental parameter dialog box**. If this window is not already open, click its button (See Figure 5-21.) in the tool bar. The experimental parameter dialog box will then appear.

3. **Change the Y experiment setting**. The Y Experiment setting will probably be set to No Y Experiment. Change this by selecting MW Power Sweep. (See Figure 5-21.)

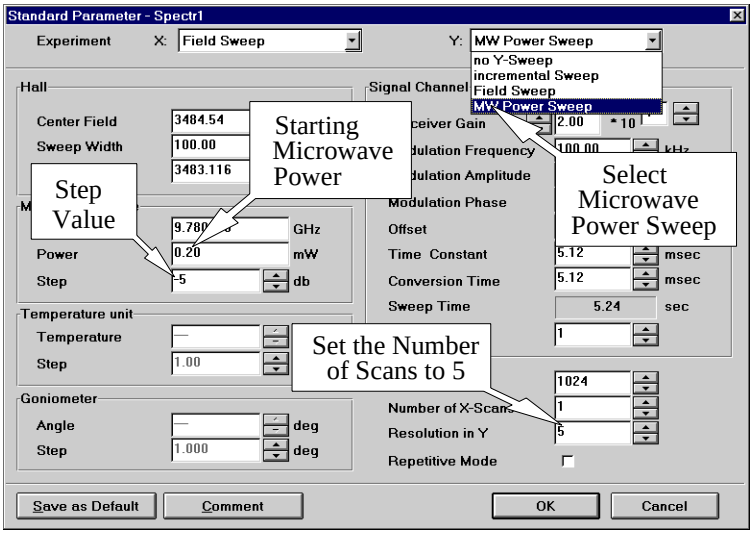


Figure 5-21 Sample parameter settings for acquiring a 2D data set.

4. **Set the starting microwave power to 0.2 milli-watts.** Change the power in the power setting box to 0.2 milliwatts. This will be the power that is used to acquire your first spectra. (See Figure 5-21.)

5. **Use a step value of - 5 dB.** By using a negative step value, the power will increase in units of 5 dB between each scan. (See Figure 5-21.)

6. **Set the number of spectra to be acquired to 5.** In the Resolution in Y box change the setting to 5. This will program the spectrometer to acquire 5 scans. (See Figure 5-21.) Click OK to close the window.

7. **Click on Run to acquire your 2D data set.** This will initiate the first of five scans with the power increasing in

units of 5 dB between each scan. You will notice the scan number updating in the box in the upper right corner of the spectrum window. (See Figure 5-22.)

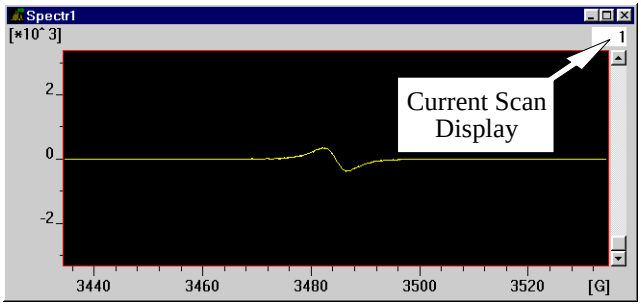


Figure 5-22 Current scan display

8. **Transfer your 2D data set to WIN-EPR.** By clicking the WIN-EPR button, you will launch the WIN-EPR program and automatically load your dataset. (See Figure 5-23.)

Figure 5-23 Launching the WIN EPR program from Win Acquisition.

9. **Display your 2D data set**. Select 2D Processing from the WIN-EPR System menu. (See Figure 5-24.) Your data should automatically appear as seen in Figure 5-25. If your data does not appear as in Figure 5-25, make sure the display mode is set to Stack Plot. (See Figure 5-26.)

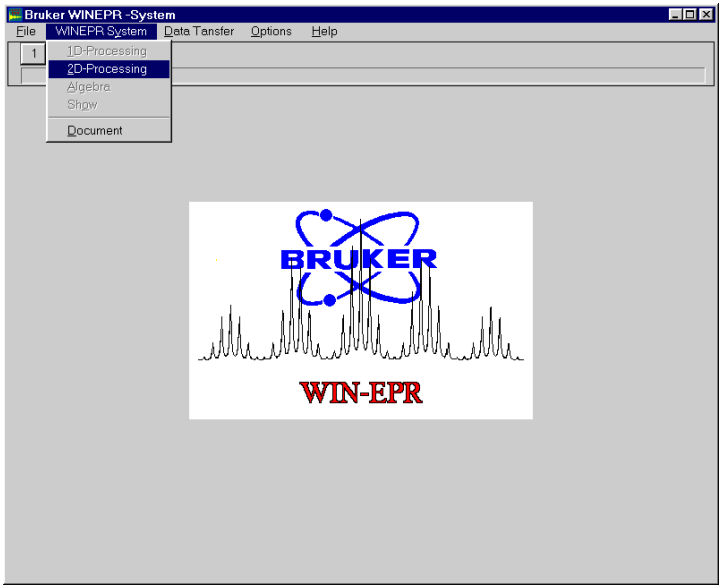


Figure 5-24 Opening a 2D dataset.

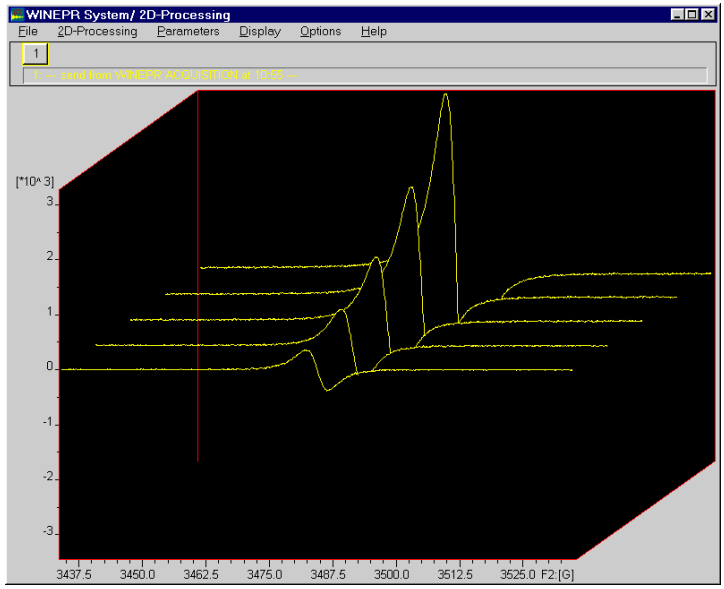


Figure 5-25 Stack plot display of 2D dataset.

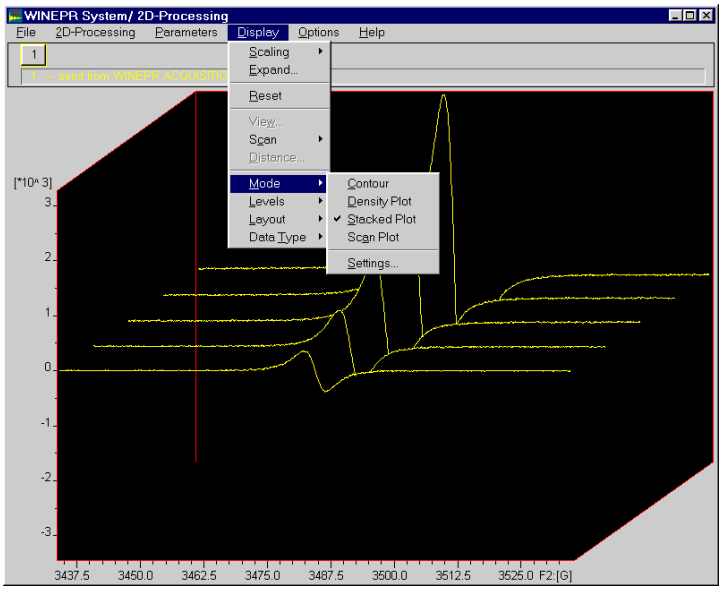


Figure 5-26 Setting the display mode to Stack Plot.

Helpful Hints 6

This chapter contains useful and helpful hints to get the most out of your EMX spectrometer and its hardware. The first half of this chapter covers advice on what to do if you do not observe an EPR signal from your sample. The second half of the chapter concerns itself with optimizing the performance of the EPR spectrometer for your particular sample and operating conditions. It is assumed that you are familiar with the material presented in Chapter 2 and Chapter 3.

Hints for Finding EPR Signals 6.1

Cryostats shifts the resonant frequency of the cavity and hence the frequency of the spectrometer to a lower value. The field for resonance of your EPR signals will therefore be lower than you would expect for a cavity without a cryostat.

- **Make sure that the spectrometer is functioning properly.** If you followed the directions of Chapter 3, this should not be a problem. There are many common mistakes. Is the modulation cable connected properly to the cavity and console? Is the waveguide gasket installed properly? Is everything turned on? Advice on troubleshooting is presented in the next chapter.
- **Scan over the correct magnetic field range.** If you do not sweep over the correct magnetic field range, you will miss your signals. This mistake occurs quite often when using a cryostat in the EPR cavity. Consult literature references to determine approximate g-values for the species in your sample. You can then choose the appropriate magnetic field for your sample. Most organic radicals will have a g-value of approximately 2. This corresponds to a field for resonance of approximately 3480 Gauss at a microwave frequency of 9.8 GHz. Metal ions can have large departures from g = 2 as well as large zero-field splittings, making it difficult to guess where the resonance might occur. Performing a wide scan in your initial experiment will maximizes your probability of finding the EPR signal.

- **Finding an EPR signal**. Sometimes you may have difficulty finding the EPR signal from an unknown sample or a sample you are not familiar with. Here we provide two examples of parameter sets that are useful for finding EPR signals from unknown samples that you suspect will consist of either an organic radical (See Figure 6-1) or a transition metal ion, (See Figure 6-2) respectively. These parameters are by no means optimized, but they will serve to help you find the signal. After you find the EPR signal you need to reset the field center and scan range. (See Section 4.3.) You also need to optimize your EPR signal using the method described later in this chapter. If you still cannot find the signal you may have to adjust parameters such as the microwave power, modulation amplitude, scan time, etc.

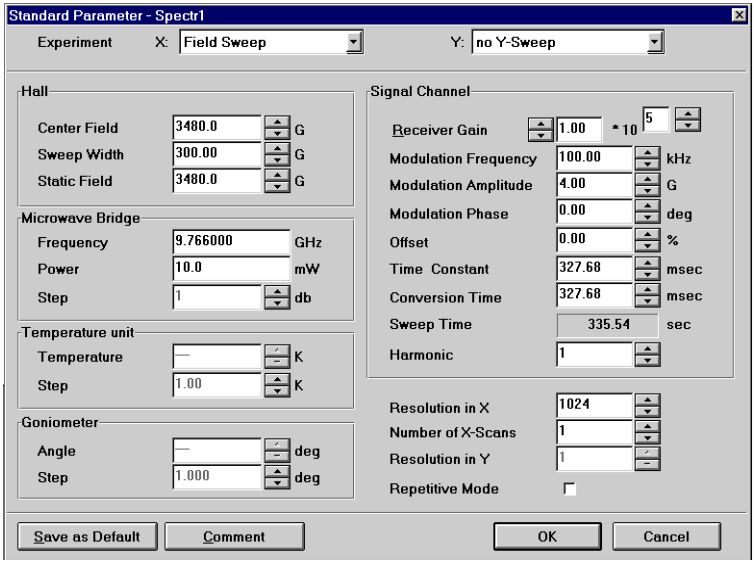


Figure 6-1 Parameters for finding an EPR signal from an organic radical.

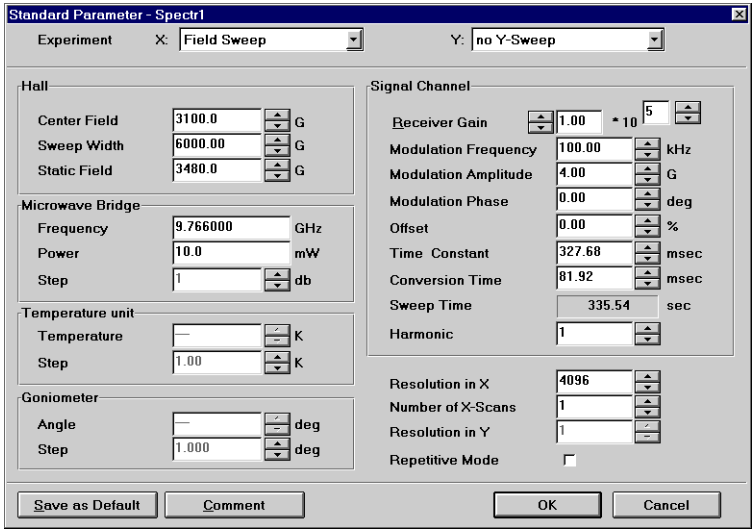


Figure 6-2 Parameters for finding an EPR signal from a transition metal ion.

- **Make sure your sample is positioned correctly in the cavity.** Only the central region of the cavity contributes significantly to the EPR signal. If you place the sample sufficiently out of this region you may not detect a signal.
- **Optimize the sensitivity.** You may have a very weak signal in which case you will need to optimize your parameter settings for sensitivity. The chart on the following page summarizes common factors that are important for getting the optimum sensitivity from your EPR measurements. The pages that follow the chart provide a more in-depth discussion of these factors.

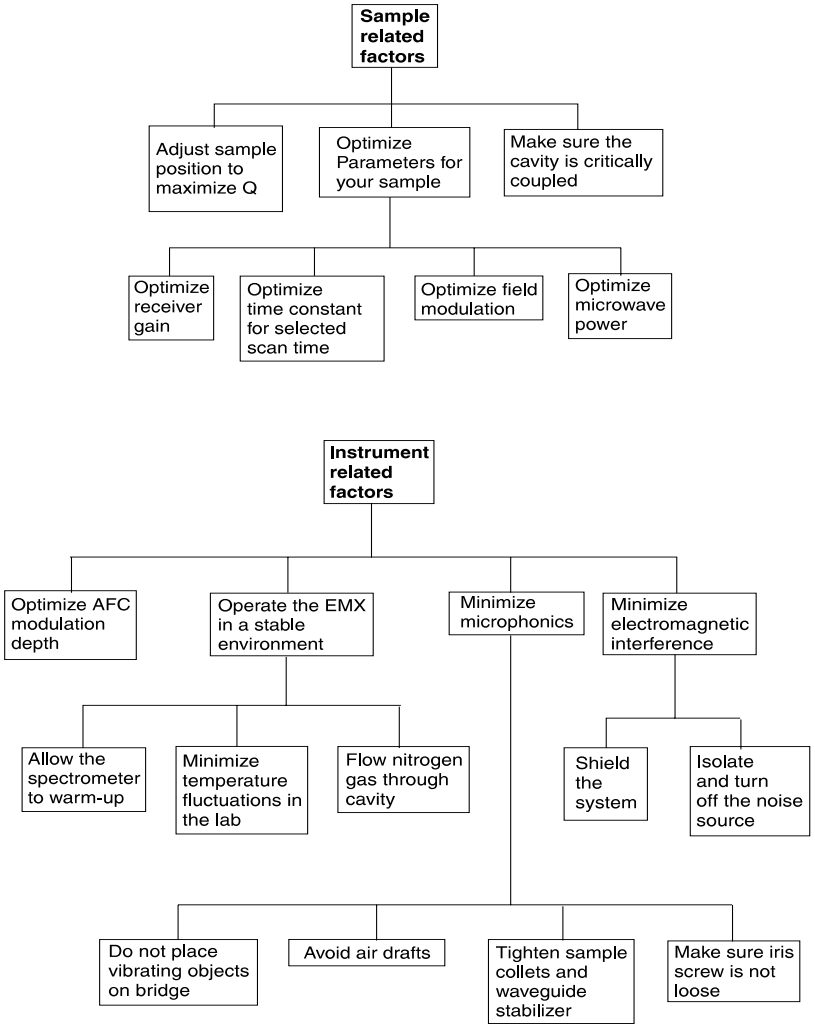

Sample related factors
Adjust sample position to maximize Q
Optimize Parameters for your sample
Make sure the cavity is critically coupled
Optimize receiver gain
Optimize time constant for selected scan time
Optimize field modulation
Optimize microwave power
Instrument related factors
Optimize AFC modulation depth
Operate the EMX in a stable environment
Minimize microphonics
Minimize electromagnetic interference
Allow the spectrometer to warm-up
Minimize temperature fluctuations in the lab
Flow nitrogen gas through cavity
Shield the system
Isolate and turn off the noise source
Do not place vibrating objects on bridge
Avoid air drafts
Tighten sample collets and waveguide stabilizer
Make sure iris screw is not loose

Figure 6-3 Factors to consider when optimizing your EMX for sensitivity.

Optimizing Sensitivity 6.2

Instrumental Factors 6.2.1

- **Minimize microphonics.** Microphonics are unwanted mechanical vibrations in the spectrometer. Depending on the nature and frequency of the microphonics, these vibrations may generate noise in your EPR spectrum. The most common microphonic sources include the cavity, the sample and the bridge. Prevent microphonic noise by securing the waveguide with the waveguide stabilizers. Rigidly secure the sample in the cavity by tightening the collets on the cavity sample stack. Do not place objects on the microwave bridge that may vibrate or are free to move. Avoid placing a frequency counter with a fan on top of the bridge.

For better spectrometer stability, keep the spectrometer away from windows and ventilation ducts.

- **Maintain a controlled environment for the best spectrometer performance.** Air drafts past the spectrometer, especially the cavity, may induce temperature fluctuations or microphonics from sample vibration. Large fluctuations in the ambient temperature may degrade performance by reducing the frequency stability of the cavity. Very humid environments may cause water condensation. You can reduce condensation inside the cavity by maintaining a constant purging stream of dry nitrogen gas. Note that excessive gas flow rates can generate microphonic noise through sample vibration.
- **Minimize electrical interference.** Noise pick-up from electromagnetic interference (EMI noise) may be encountered in some environments. You may be able to minimize EMI noise by shielding or perhaps by turning the noise source off if generated by equipment near the spectrometer. There is often less EMI at night.

- **Allow the spectrometer to warm-up.** One hour is usually adequate to achieve a stable operating temperature. For maximum stability under extreme operating conditions such as any combination of high microwave power, high magnetic field modulation amplitudes, and variable temperature work, allow the system to equilibrate under the same conditions as the experiment will be performed.
- **Carefully follow the procedure for positioning the sample inside the cavity.** This is particularly important for samples exhibiting a large dielectric loss. Improper sample positioning can perturb the microwave field mode patterns in the cavity, resulting in less than optimum sensitivity.
- **Periodically check the iris coupling screw for tightness of fit.** A worn iris screw thread will make the iris susceptible to microphonics which can modulate the cavity coupling.
- **Critically couple the cavity.** Best cavity performance is obtained with a critically coupled cavity. Maximum transfer of power between the cavity and the waveguide occurs under this condition.
- **Optimize the AFC.** Adjust the AFC modulation depth to minimize the noise level observed in the absorption EPR spectrum at full incident microwave power. Adjustments of the AFC MOD LEVEL potentiometer, located on the rear of the microwave bridge, (Figure 6-4) should be made while in the Operate mode with the sample inserted and the spectrometer tuned as described in Section 3.4. You should make this adjustment for all experiments limited by signal to noise considerations. The optimum AFC modulation depth is a function of the loaded cavity Q. Consequently, slight variations in the optimum setting may be anticipated. If you are using a finger dewar with a boiling refrigerant such as liquid

nitrogen, you should turn the AFC modulation level to maximum.

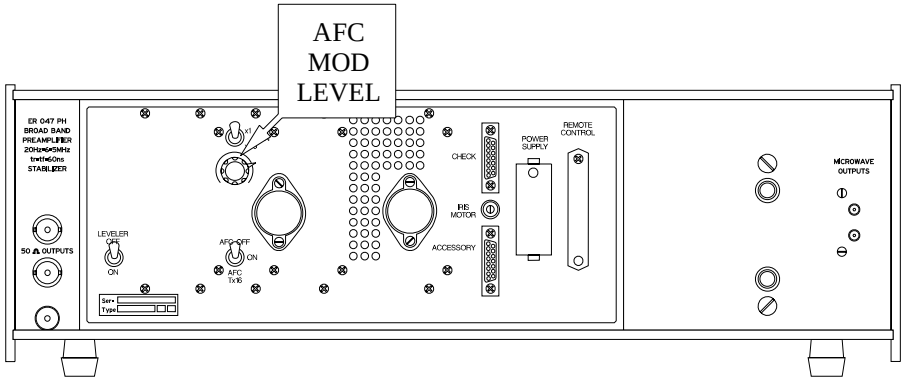


Figure 6-4 Location of the AFC MOD LEVEL potentiometer

Cryostats can protect your cavity from contamination due to sample tube breakage.

- **Insert a cryostat in the cavity.** Quartz has a dielectric constant of 3.8 but a low dielectric loss. Inserting high purity quartz sleeves, such as the variable temperature dewar, actually concentrates the microwave magnetic field intensity at the sample. The increased field intensity produces an EPR signal that has a larger signal to noise ratio than is achieved in the absence of the dewar insert. If your experiments approach the sensitivity limit and your samples are nonlossy you may benefit from the use of the variable temperature quartz insert dewar, even if the experiment is run at room temperature.

Parameter Selection 6.2.2

- **Optimize the receiver gain.** You need to have sufficient receiver gain in order to see all the details in your spectrum. Figure 6-5 shows the results of insufficient as well as excessive receiver gain. If the receiver gain is too low you will see the effect of digitization in the spectrum (spectrum b), whereas at high gain the signals will clip due to an overload in the signal channel (spectrum c). A good way to automatically optimize your receiver gain is to use the set field center and field range button in the tool bar as described in Section 4.3.1. When you draw a rectangle around the entire spectrum, the receiver gain is automatically set such that the newly acquired spectrum will fill the display completely.

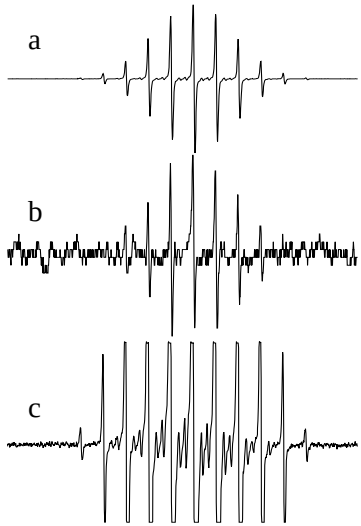


Figure 6-5 Effect of using gain settings that are either (a) optimal, (b) too low, or (c) too high on an EPR spectrum.

- **Optimize the conversion time.** The conversion time you select will affect the dynamic range of your experiments. The conversion time is actually the amount of time the analog-to-digital converter spends integrating at one field position before moving to the next field value in the sweep. If you need to resolve lines that are very intense as well as lines that are very weak (i.e, carbon 13 satellites) within the same spectrum you will need to use a sufficiently long conversion time. If the conversion time is too short the smaller signals will be lost in the steps of the digitizer. The conversion time you select will also determine the sweep time. That is, the sweep time will be equal to the conversion time multiplied by the number of data points in the spectrum. (See selecting the number of data points below.)

- **Optimize the time constant for the selected conversion time.** The time constant filters out noise; however, if you choose a time constant that is excessively high relative to your sweep time, you may actually filter out your signal! You should adjust your time constant to "fit" the conversion time you have selected. These two parameters are actually very related because the conversion time will determine the total sweep time. You need to use a time constant that will be sufficiently long to filter out undesirable noise yet short enough that you do not distort your signal. Therefore, if you want to use a longer time constant you will need to increase the scan time as well. Figure 6-6 shows the effect of progressively increasing the time constant while maintaining the same sweep time. All the spectra are at the same scale. A safe rule of thumb is to make sure that the time needed to scan through an EPR signal (*i.e.* one EPR line) is ten times greater than the length of the time constant. A time constant that is 1/4 that of the conversion time will guarantee that your spectrum is not distorted. However, for samples limited by a low signal to noise ratio, you may want to make the time constant equal to the conversion time or greater.

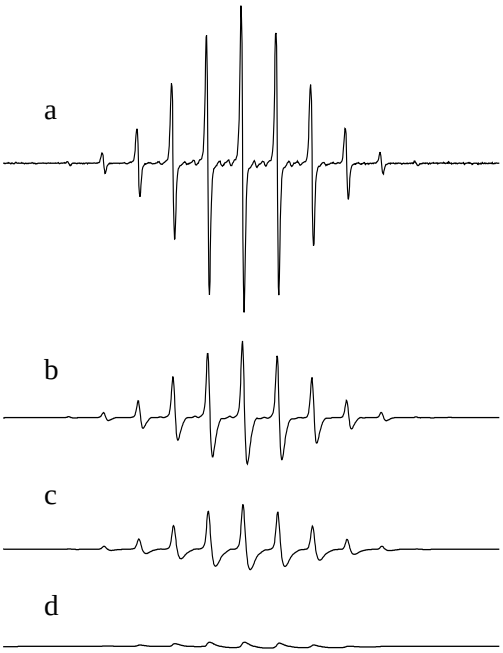


Figure 6-6 Effect of using a progressively longer time constant (a-d) on an EPR spectrum.

- **Selecting the number of data points.** The number of data points is the other parameter that will determine the appropriate sweep time. A general rule is to make sure that you have at least 10 data points within the narrowest line that you are trying to resolve. This means that for EPR signals with very narrow lines you will need to increase the number of data points that are collected for a given field sweep. However, if the lines of your EPR signal are sufficiently wide, increasing the number of data points will not yield any additional information, but will only result in longer sweep times. With the EMX you can select 512, 1024, 2048, 4096 or 8192 data points. Remember, you will probably want to increase

the time constant by a factor of two as you double the number of data points. Figure 6-7 shows the enhancement in resolution achieved by increasing the number of data points.

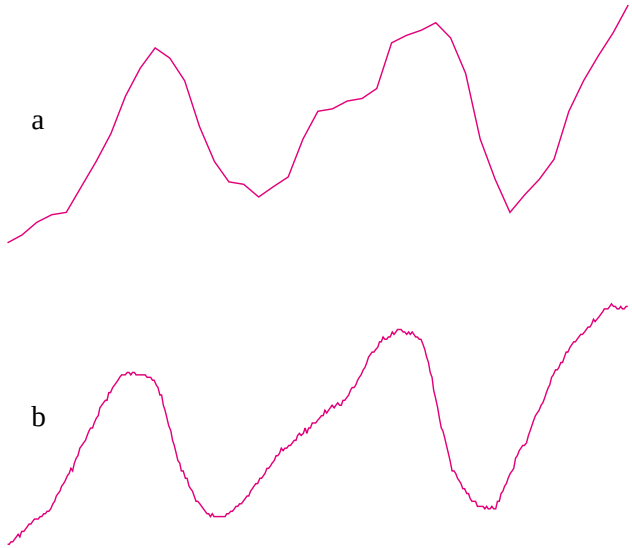


Figure 6-7 Expanded view of narrow lines in an EPR spectrum using 1024 points (a) or 8192 points (b).

- **Optimize the field modulation amplitude.** Excessive field modulation broadens the EPR lines and does not contribute to a more intense signal. Figure 6-8 shows the results of excessive field modulation. You can see how some of the smaller lines in spectrum a were lost in spectrum b even after increasing the modulation only slightly. A good “rule of thumb” is to use a field modulation that is approximately the width of the narrowest EPR line you are trying to resolve. Keep in mind that there is always a compromise that must be made between resolving narrow lines and increasing your

signal to noise ratio. If you have a very weak signal, you may need to sacrifice resolution (*i.e.*, by using a higher field modulation) in order to even detect the signal. However, if you have a high signal to noise ratio, you may choose to use a much lower field modulation in order to maximize resolution.

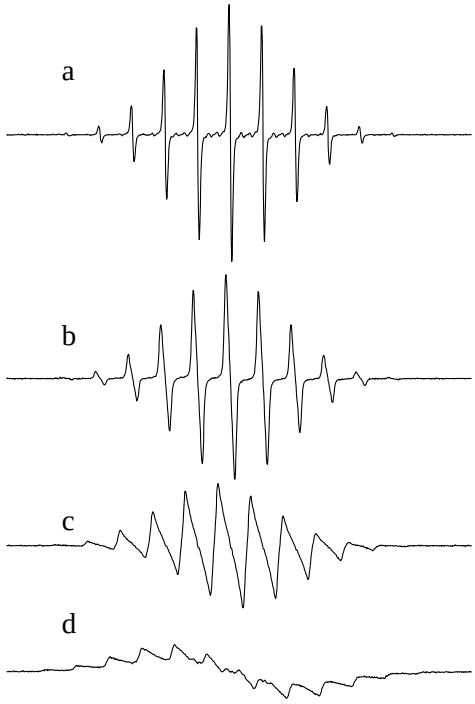


Figure 6-8 Effect of using progressively higher field modulation (a-d) on an EPR spectrum.

- **Optimize the microwave power level.** The intensity of an EPR signal increases with the square root of the microwave power in the absence of saturation effects. When saturation sets in, the signals broaden and become weaker. EPR signals with very narrow lines are particularly susceptible to distortion by excessive power. Figure 6-9 shows the result of excessive microwave power. You should try several microwave power levels to find the optimal microwave power for your sample. A convenient way to find the optimum power is to use the 2D experiment routine described in Section 5.4.

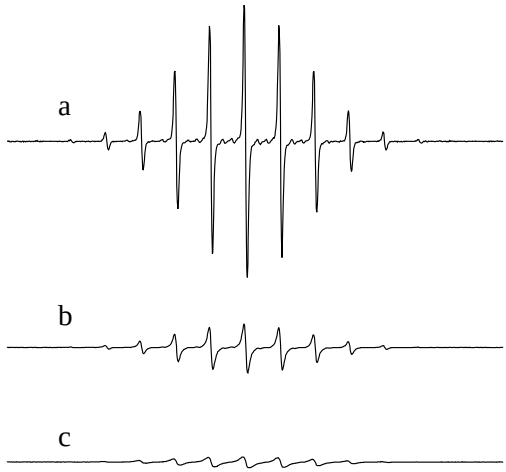


Figure 6-9 Effect of using progressively higher power (a-c) on an EPR spectrum.

- **Signal averaging.** With a perfectly stable laboratory environment and spectrometer, signal averaging and acquiring a spectrum with a long scan time and a long time constant are equivalent. Unfortunately, perfect stability is usually impossible to attain and slow variations can result in considerable baseline drifts. A common cause of such variations are room temperature changes or air drafts around the cavity. For a slow scan, the variations cause broad features to appear in the spectrum as shown in spectrum b of Figure 6-10. You can achieve the same sensitivity without baseline distortion by using the signal averaging routine with a small time constant and shorter scan time. For example, if you were to signal average the EPR spectrum using a scan time that was significantly shorter than the variation time, these baseline features could be averaged out. In this case, the baseline drift will cause only a DC offset in each of the scanned spectra. Spectrum a shows the improvement in baseline stability through the use of short time scans with signal averaging when the laboratory environment is not stable.

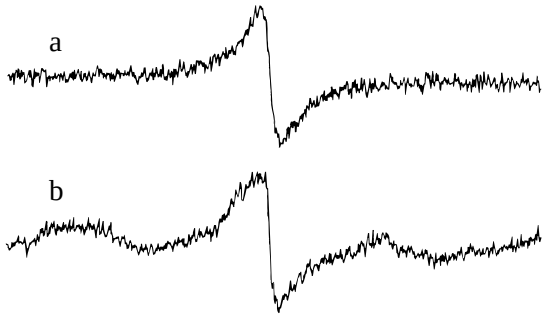


Figure 6-10 a) Signal acquired with short time sweeps and signal averaging.

b) Signal acquired with long time sweep and long time constant.

Troubleshooting 7

This chapter lists some common problems you may encounter with your Bruker EMX EPR spectrometer. Major hardware malfunctions are not covered. We concentrate on problems due to operator errors, set up errors, or protective circuitry. The material presented in Chapters 2, 3, and 4 is useful in understanding much of what is discussed in this chapter. Many problems are easily solved by the user. The flow diagram on this page will help you diagnose the majority of problems that occur during the tuning phase of operation. If you fail to find a solution to your problem after reading this chapter, call your local Bruker EPR service representative.

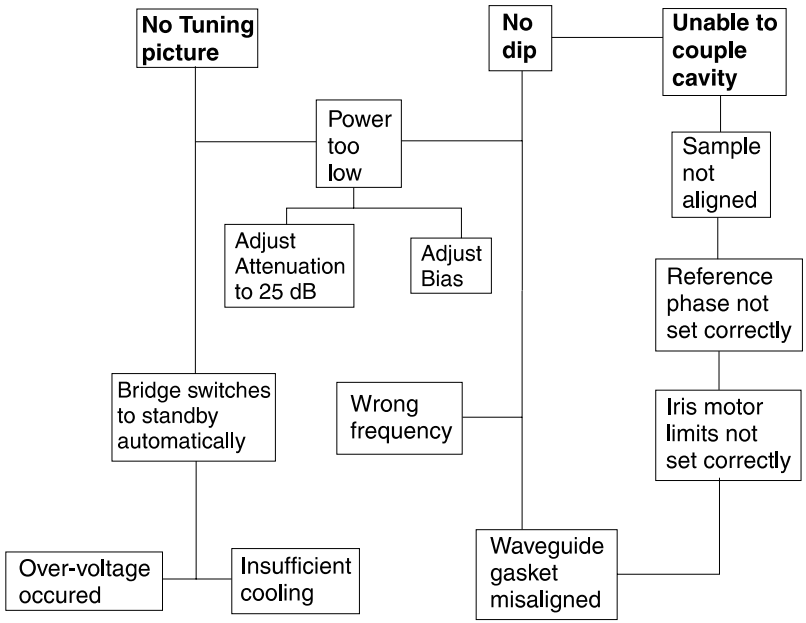


Figure 7-1 Flow Chart for diagnosing problems.

... not ready! 7.1

- If a warning dialog box appears when you first start the Acquisition program with a message such as Field Controller not ready! or Signal Channel not ready!, you have probably forgotten to turn the console power supply on.

No Cavity Dip. 7.2

- **Waveguide gasket installed improperly.** See Figure 5-12 for the proper orientation of the gasket.
- **Cavity undercoupled or overcoupled.** First, look at the microwave frequency where you normally expect the cavity to resonate and then adjust the iris screw for better coupling. This can occur when working with lossy samples such as aqueous solutions in flat cells or capillaries.
- **You need more microwave power.** If you are using insufficient microwave power, it can be difficult to see the cavity dip. We recommend setting the microwave attenuator at 25 dB for the best visibility.
- **You are not at the correct frequency.** By putting the sample in, you will cause the cavity to resonate at a lower frequency. Thus, you will usually need to lower the frequency after you have placed the sample in the cavity in order to see the dip.

Tuning Error 7.3

Both the auto-tune and fine-tune procedures of the microwave bridge controller will terminate with an appropriate error message if a particular parameter cannot be set or optimized. Here are the possible error messages.

- **Tuning Frequency.** Both the upper and lower limits of the frequency range (i.e., 8.9-9.9 GHz) have been reached and no defined dip has been detected. Check manually if a dip can be found. A very slight dip (e.g. very lossy sample) may not be detected by the auto-tune routine.
- **Adjusting Ref. Arm Phase.** The full 360° range of the signal phase has not resulted in an optimal phase setting.
- **Adjusting Ref. Arm Bias.** The system is unable to set the diode current to 200 microamperes at 50 dB attenuation.
- **Adjusting AFC Lock Offset.** The system is unable to set the AFC lock offset to zero. Check the back of the bridge to make sure the AFC is on. If this error occurs during fine-tune, try auto-tune.
- **Critically coupling cavity.** The iris motor has reached both of its limit switches and has been unable to obtain a diode current of 200 microamperes. Check if the iris motor is still connected to the screw and that the limit switches have been set properly. (See Section 5.2.) If you are using a flat cell when this happens, it is likely that you need to adjust the position of the flat cell. It is easier to optimize the cavity dip if you adjust the flat cell while you are looking at the tuning picture. If this error occurs during fine-tune, try auto-tune.

No Tuning Picture 7.4

- **Tune mode delay period not expired (klystron bridge only).** After you turn on the spectrometer, a delay of approximately three minutes is required before a klystron will activate as you switch from Stand By to Tune. This does not apply to Gunn diode bridges.
- **Reference microwave power too low (klystron or Gunn diode bridge).** Carefully adjust the Bias slider bar of the Microwave Bridge Control dialog box until you observe a tuning mode pattern on the display.
- **Microwave bridge controller automatically switches from Tune to Stand By (klystron or Gunn diode bridge).** There is insufficient cooling for the microwave source. The protection circuitry will shut the microwave source off if the temperature rises too high. Make sure that the valves for the coolant lines leading to the bridge are open. (See Section 3.2.) Make sure that the heat exchanger is on and has sufficient water flow.
- **Microwave bridge controller automatically switches from Tune to Stand By. (klystron bridge only).** There is protection circuitry which protects the microwave source from voltage spikes. To reset the protection circuitry, turn the console power off for approximately three seconds and turn it on again. The voltages used in the Gunn diode bridge are not sufficiently high to require this type of protection circuitry.

Unable to Critically Couple Cavity 7.5

- **Sample position.** If too much of a lossy sample is in the microwave electric field in the cavity, you will not be able to critically couple the cavity. Move the sample until the coupling becomes better. The sample position is particularly critical for flat cells and capillaries.
- **Microwave reference phase.** If the microwave reference phase is not set properly, you will not be able to critically couple the cavity. Carefully follow the instructions in Section 3.4 when tuning the spectrometer.
- **Iris motor limits improperly set.** If the iris motor limits were improperly set, the iris can not be screwed in sufficiently. Follow the procedure in Section 5.2 to properly adjust the iris motor limits.
- **Iris tip size.** When working with lossy samples, it is advisable to use a larger iris tip to increase the coupling range of the cavity. This is particularly important when working with flat cells or capillaries. Contact your Bruker service representative for advice.

Magnet Power Supply Shuts Down 7.6

- **Insufficient cooling capacity.** Make sure that the heat exchanger is on and that there is sufficient cold water flowing through it. Either the Ext. or Temp. warning LED's on the magnet power supply will light up with this fault.
- **Hall probe inserted with the wrong polarity.** The magnetic field will go to the maximum field.
- **Hall probe fallen out of the magnetic air gap.** If the Hall probe has fallen from the pole piece of the magnet, the power supply may go to the maximum current value.

Baseline Distortion 7.7

- **Linear baseline drifts.** The use of very large modulation fields can produce large eddy currents in the cavity side walls. These currents can interact with the magnetic field to produce a torque on the cavity and create a resonant frequency shift. A linear field dependent or modulation amplitude dependent baseline is indicative of such an effect. This phenomenon should not be observed if the cavity end plates are properly fitted and torqued. Do not attempt to adjust the torque on the plates. Contact your local Bruker EPR service representative.
- **Slowly and randomly varying baseline.** The use of high microwave power or large modulation fields can heat the cavity and the sample. The ensuing thermal drifts in the coupling of the cavity, as well as the frequency of the cavity, can result in a fluctuating offset in the signal. Allow the tuned cavity and sample to come to thermal equilibrium before performing the final tuning of the cavity. Once the cavity is equilibrated and properly tuned under the equilibrated condition, you can start acquiring a spectrum. Avoid air drafts around the cavity, as they can randomly change the temperature of the cavity and sample and hence, the baseline of the spectrum.

- **Variable temperature operation.** Cavity frequency and coupling instability may be induced during variable temperature operation, especially at very low or very high temperatures. Increase the flow rate of the cavity and waveguide purging gas as the operating temperature departs further from room temperature. Wait for the cavity to stabilize at each new operating temperature before recording the spectrum. Retune the cavity to compensate for any frequency shift and re-establish critical coupling at each temperature.
- **Background signal.** Your cavity, cryostat, sample tube, or sample may be contaminated. Call your local Bruker EPR Service representative for advice. Never take the cavity apart to clean it.

Excessive Noise Output 7.8

- **Electromagnetic interference.** Verify that laboratory equipment is not a source of electromagnetic interference (EMI). If possible, turn off all other equipment in the laboratory and observe spectrometer noise output. Determine if radio, microwave, or TV broadcasting stations are operating in proximity to the spectrometer. Record the noise level while operating at various times of the day and night. EMI related noise will often be reduced at night.
- **Power line noise.** Check the noise content of the AC power lines feeding the spectrometer. Line transients or momentary blackouts will drastically degrade the performance of high gain detection systems such as EPR spectrometers.
- **Ground loops.** Ground loops are very common and often difficult to avoid. Disconnect accessory equipment, especially if it is plugged into remote AC outlets and observe the noise level. Turn off the magnet power supply and observe the noise level. If the noise level changes during either of these tests, consult your local Bruker EPR service representative for alternate installation planning.

- **Microphonic generated noise.** Secure the waveguide and cavity assembly by using the plastic waveguide stabilizers. Secure the sample firmly in the collet. If you use a cryostat, make sure that the cryostat sits firmly in the cavity. Make sure that an excessive nitrogen gas flow rate through the cryostat does not vibrate the sample.
- **Worn iris screw.** Check for a worn iris coupling screw. An iris screw that does not fit snugly in the waveguide may generate noise by modulating the cavity coupling. Replace the worn iris screw with a new one.
- **Boiling liquids.** If you are using a dewar with a boiling refrigerant such as liquid nitrogen, you will need to increase the AFC modulation level.

Poor Sensitivity 7.9

- **Excessive microwave power.** The microwave power may be set too high, which will cause your sample to saturate. Optimize the power for your sample by recording spectra at a variety of power levels.
- **Wrong cavity type for sample.** The type of cavity you use for a particular sample can make a large difference in sensitivity. Consult the Bruker literature on the full line of EPR cavities to determine which one is best for your samples.
- **Low cavity Q.** The cavity Q can be degraded because of improper sample positioning. Having your sample positioned in the microwave electric field will reduce the sensitivity by degrading the cavity Q, especially for samples with high dielectric loss. This can happen if you are using flat cells or capillaries. Observe the Q value read-out in the microwave bridge dialog box when you are adjusting the sample position.
- **Cavity not critically coupled.** Maximum power is transferred between the cavity and waveguide when the cavity properly matches the impedance of the waveguide, (*i.e.*, is critically coupled.). A drastically undercoupled iris will not transmit power to the cavity and so will not excite EPR transitions. A drastically overcoupled cavity will have a lower Q, resulting in lower sensitivity. These effects can occur when using lossy samples such as aqueous solutions or conducting samples.

- **Water condensation.** During low temperature operation, water can condense inside the cavity. Water, being a high dielectric loss material, will absorb the microwave power in the cavity and destroy the cavity Q. Avoid condensation by using a purging nitrogen gas flow through the cavity.
- **Signal channel not calibrated.** The modulation amplitude and phase of the signal channel may not be properly calibrated. Make sure that you load the proper calibration file into the data system. Also, make sure that the Calibrated check button in the Interactive Spectrometer Control dialog box is not un-checked.
- **Receiver gain or modulation not optimized.** See Section 6.2.2.
- **Sample not positioned properly.** Center your sample in the cavity.

Poor Resolution 7.10

- **Microwave power set too high.** Saturating microwave power levels will broaden your resonance line. Verify that the linewidth is independent of the microwave power level by recording the spectrum at various power levels.
- **Modulation amplitude set too high.** Large field modulation amplitudes will broaden your resonance line, particularly as the modulation amplitude approaches the linewidth. Reduce the modulation amplitude to ensure that the spectrum is independent of the modulation amplitude. (See Figure 6-8.)
- **Modulation frequency set too high.** The spectral resolution is limited by the field equivalence of the modulation frequency used. Reduce the modulation frequency to verify that the linewidth is independent of the frequency. (See Figure 2-17.)
- **Time constant too long for sweep time.** A larger time constant will begin to filter out the high frequency components of your signal. Consequently, if the sweep rate is too fast relative to the time constant, the spectrum will appear distorted and broadened. To avoid this problem make sure that the time required to sweep through one of your EPR lines is at least ten times the length of the time constant. (See Figure 6-6.)

- **Magnetic field inhomogeneities or gradients.** Extremely narrow lines, less than 20 milliGauss, may be limited by magnetic field irregularities. Vary the position of the cavity in the magnet air gap. If the linewidth changes, check for magnetic objects in or around the magnet. If possible, suspend these objects by a string and watch for a deflection in the same field strength as used in the experiment. Do not attempt this with the cavity in the magnet. The force of a ferromagnetic object being pulled into the magnet air gap can cause serious damage to accessories in the air gap.
- **Spectrometer not thermally stabilized.** Be sure that the spectrometer has been turned on for several hours. Verify that the laboratory conditions are within specified limitations, *i.e.*, temperature fluctuations, *etc.*

Lineshape Distortion 7.11

- **Microwave power too high.** The effect of saturating microwave fields is to broaden the resonance. This is easily apparent for single structureless lines; however, small splittings may become unresolvable if strongly saturating levels of microwave power is used. Lower the microwave power until you obtain a power independent lineshape.
- **Modulation amplitude too high.** Large field modulation will broaden the resonance line. Lower the modulation amplitude to a region where the lineshape is independent of the modulation amplitude. (See Figure 6-8.)
- **Time constant too long for sweep time used.** A safe rule of thumb is that the time required to sweep through an EPR line should be ten times the length of the time constant. (See Figure 6-6.)
- **Modulation frequency too high.** The modulation frequency can determine the resolution of the experiment. The spectral profile may also change, due to the effect of molecular dynamics, if saturating microwave fields are applied. These effects are especially pronounced if the motional frequency for the spin dynamics is similar to the applied modulation frequency. The technique of saturation transfer is based on this mechanism. The spectral profile may change markedly if the modulation frequency is varied while applying strong microwave fields. (See Figure 2-17.)
- **Magnetic field gradients.** These may produce highly asymmetric lineshapes. Reposition the cavity within the magnet air gap to check the magnet for homogeneity. Check for magnetic objects in or around air gap. Magnetic field inhomogeneity could also broaden the response to obscure splittings by overlapping spectral components.

- **Anisotropic g matrix.** A highly anisotropic g-matrix naturally produces asymmetric lines.
- **Background signal.** A strong background signal from contamination of the EPR cavity or the sample can distort your EPR spectrum.
- **High conductivity.** High conductivity exhibited by samples with mobile electrons will result in asymmetric lines known as Dysonian lineshapes. This results from a mixing of absorption and dispersion components induced in the sample itself.
- **Lossy samples.** If you put large lossy samples in a cavity, you can also obtain Dysonian lineshapes. Use progressively smaller capillaries until you obtain a symmetric lineshape.
- **Microwave reference phase.** The dispersion signal from easily saturated samples can be very large compared to the absorption signal. To minimize the contribution of the dispersion signal, carefully adjust the microwave reference phase. In addition, make sure that the AFC offset is close to zero.
- **Magnetic field drifts.** Magnetic field drift may produce an asymmetric or distorted line for samples exhibiting very narrow resonance linewidths. This problem may arise for linewidths less than 20 mG. Use a field-frequency lock system to eliminate field drift problems.

No Signal When Everything Works 7.12

- **Check cables.** Make sure that all the cables are connected. Check the modulation cable and the preamplifier cable.
- **Sample position.** If you have a small sample, make sure that the sample is centered in the cavity.
- **Magnetic field values.** Are you using the correct field values to see your EPR signal? If you are using a cryostat, remember that the microwave frequency drops and hence the field for resonance will also be lower. Is the Hall probe positioned properly in the magnet?

Warning Noises 7.13

- **High pitched noise from the heat exchanger.** The heat exchanger will emit a high pitched noise when it requires more distilled and deionized water.
- **Funny noises from the iris motor.** Stop turning the iris motor immediately. You may be breaking the iris screw.

EPR Spectrometer Calibration 8

For many experiments, it is vital that your spectrometer is carefully calibrated. For example, it is essential to know the precise values of the magnetic field modulation amplitude in order to obtain quantitative EPR spectra. The calibration procedures in this chapter enable you to measure the experimental conditions produced by the spectrometer with considerable accuracy.

This chapter is not meant to be a general overview of spectrometer calibration and quantitative EPR. Therefore, we highly recommend the following references which discuss the topic in much greater detail:

- Poole, C.P. *Electron Spin Resonance, a Comprehensive Treatise on Experimental Techniques:* First Ed., Interscience, New York, 1967.
- Poole, C.P. *Electron Spin Resonance, a Comprehensive Treatise on Experimental Techniques:* Second Ed., Wiley, New York, 1983.
- Alger, R.S. *Electron Paramagnetic Resonance:* Interscience, New York, 1968.

Standard Samples 8.1

Standard samples are useful for system performance tests, spectrometer calibration, and quantitative concentration measurements. Ideally the standard sample should contain stable, long lived paramagnetic species, be easily prepared under consistent and controlled methods, and should be fully characterized with respect to all spectroscopic parameters such as relaxation times and hyperfine and fine structure splittings. In addition, the resonance line should be narrow and preferably homogeneous. Unfortunately, the universal standard sample has not been found. Many standards have been suggested and each has its own particular merit. The standard samples supplied with every Bruker spectrometer are discussed below.

DPPH (α, α‘ - diphenyl-ß-picryl hydrazyl) 8.1.1

DPPH serves as a reference both in the solid state and in the liquid state when dissolved in benzene or toluene/mineral oil. The line width measured from the solid is subject to exchange narrowing and thus, varies from under 1 gauss to over 4 gauss, depending on the solvent that was used for recrystallization. It has a g factor of 2.0036 ± 0.0003. When dissolved in solution, a quintet with unresolved hyperfine couplings is observed as the spin exchange narrowing is reduced as the sample is diluted. A small single crystal of DPPH is an ideal sample for calibrating the phase and the field modulation amplitude of the signal channel of an EPR spectrometer. DPPH has been studied extensively by:

- Möbius, K. and R. Biehl. *Multiple Electron Resonance Spectroscopy:* Plenum Press, 1979.
- Dalal, N.S., D.E. Kennedy, and C.A. McDowell. *J. Chem. Phys.:* **59**, 3403 (1979).

- Hyde, H.S., R.C. Sweed, Jr., and G.H. Rist. *J. Chem. Phys.:* **51**, 1404 (1969).
- Dalal, N.S., D.E. Kennedy, and C.A. McDowell. *J. Chem. Phys.:* **61**, 1989 (1974).
- Dalal, N.S., D.E.Kennedy, and C.A. McDowell. *Chem. Phys. Lett.:* **30**, 186 (1975).

Weak and Strong Pitch Samples 8.1.2

Pitch in KCl has emerged as a standard because of its long-lived paramagnetic radicals and low dielectric loss. Because of the long life of the radicals, it is unsurpassed as a test of spectrometer sensitivity. The pitch is added to a powder of KCl and the mixture is carefully mechanically mixed to obtain a homogeneous sample. After mixing, the sample is heated, pumped and sealed under vacuum. Pitch is generally prepared in two concentrations: strong pitch which is 0.11% pitch in KCl, and weak pitch which is 0.0003% pitch in KCl.

To correct for variations in spin concentration, each weak pitch sample is compared to a “standard” and assigned a correction factor. The peak to peak line width is typically 1.7 G with a g-factor of 2.0028. The size (very weak) of the signal renders pitch ill suited for modulation amplitude calibration. The weak pitch samples from Bruker Instruments have a nominal concentration of 10^{13} spins per centimeter. The samples are calibrated and the correction factor is printed on the side of the tube. This sample is prepared for the purpose of measuring instrument performance owing to its high stability, however, it is not meant as a quantitative spin-counting standard.

Calibration of the Signal Channel 8.2

You need to carefully calibrate your spectrometer's signal channel reference phase and modulation amplitude in order to obtain maximum sensitivity, minimum distortion, and quantitatively reproducible measurements. The EMX027 in conjunction with the WIN-EPR Acquisition software make this calibration easy to perform. The results of the calibration are saved on disk for future use. We recommend recalibration at least once a year to ensure quantitative and reproducible results. Each cavity or resonator has its own individual calibration file, therefore, this procedure must be followed for each cavity.

Basic Theory 8.2.1

Calibration of the signal channel involves two separate yet interdependent procedures. The first procedure is to calibrate the peak to peak modulation amplitude. For the sake of brevity, modulation amplitude will be used in place of peak to peak modulation amplitude. The second procedure is to calibrate the phase difference between the reference signal and the modulated EPR signal. Because the calibration and adjustment of the modulation amplitude can affect the phase difference, the first procedure is performed first.

You calibrate the modulation amplitude by overmodulating a narrow EPR signal. A crystal of DPPH, with a line width of approximately 1 G, is a very good sample to use. When the modulation amplitude is large compared to the line width, the magnetic field modulation brings the sample into resonance before and after the magnet has reached the field for resonance. This results in a broadening and distortion of the EPR signal. (See Figure 8-1.) In the limit of an infinitesimally narrow EPR signal, the peak to peak width of the first derivative EPR signal will be approximately equal to the peak to peak modulation amplitude.

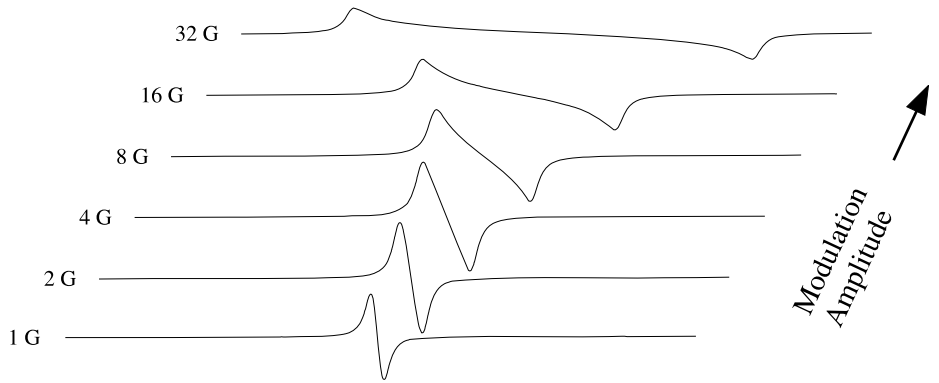


Figure 8-1 The signal shape of the DPPH EPR signal as a function of the field modulation amplitude.

The first step of calibrating the modulation amplitude involves choosing the correct tuning capacitors. The modulation amplifier needs a bit of help to obtain large modulation amplitudes at modulation frequencies greater than 50 kHz. This is a consequence of the decreasing skin depth with increasing frequency. The modulation coils on the cavity are tuned, or made resonant, by adding a tuning capacitor in series with the modulation coil.

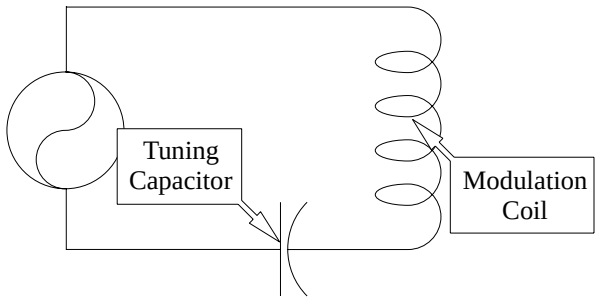


Figure 8-2 The LC resonant circuit for high frequencies.

The calibration routine switches various tuning capacitors in and out of the circuit until the modulation amplitude is maximized. The optimal capacitor for that particular frequency as well as the modulation amplitude for full gain of the modulation amplifier are recorded and saved with the calibration file. Once this data is available, the signal channel will then vary the input signal to the modulation amplifier to produce the modulation amplitude that you have selected.

Once the modulation amplitude has been calibrated, the reference phase is easily calibrated by studying the phase angle dependence of the signal intensity. The intensity of the output signal is proportional to the cosine of the phase difference between the reference signal and the modulated EPR signal. (See Figure 8-3.) It is most convenient to determine where the 90° phase difference occurs because first, the absence of a signal ($\cos(90°) = 0$) is easy to detect and second, the cosine function (and hence the intensity) changes rapidly with respect to the phase angle at 90°. In the calibration routine, spectra are acquired at several different values of the reference phase and the 90° phase difference is extrapolated from the signal intensities. The phase angle resulting in maximum signal intensity for that particular frequency is recorded and saved with the calibration file.

The phase difference between the modulated EPR signal and the reference signal depends on several experimental conditions. The length of the cable leading to the modulation coils, the inductance of the coils in the particular cavity, the gain setting of the modulation amplifier, the tuning capacitors, and the signal channel used can all change the phase difference. However, the reference phase calibration is performed automatically during the routine described in this section.

The two editions of the book by C.P. Poole that are mentioned at the beginning of this chapter are very good references for the details on the theory of phase sensitive detection and the calibra-

tion of signal channels. We encourage you to explore this topic further to learn more about calibration.

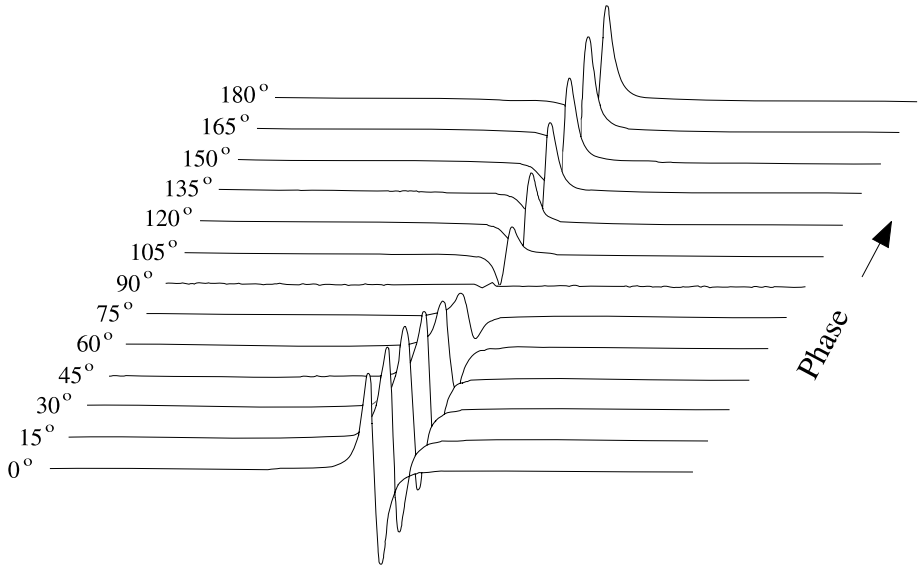


Figure 8-3 Signal intensity as a function of the reference phase angle.

Preparing for Signal Channel Calibration 8.2.2

Do not attempt to calibrate a cavity with an ER 4112HV or ER 4113HV helium cryostat installed in the cavity.

1. **Follow the instructions of Sections 3.2 through 3.5 of this manual.** You should have the spectrometer turned on, the cavity properly installed with a Bruker standard DPPH sample in it, and the microwave bridge and cavity tuned. Remove cryostats from the cavity because it is easier to position the DPPH sample properly in the cavity. (Except for the FlexLine resonators: it is necessary to use the ER 4118CF cryostat when calibrating FlexLine resonators.) In particular, the ER 4112HV and ER 4113HV helium cryostats prevent the correct positioning of the sample. Another advantage is that the resonant

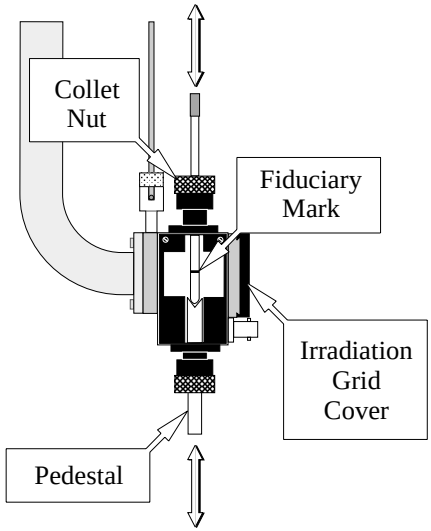


Figure 8-4 Proper positioning of the DPPH sample.

frequency of the cavity will be approximately 9.8 GHz without the helium cryostat and the field for the DPPH signal will be known (approximately 3480 Gauss). The

DPPH sample is a small point sample and therefore has a fiduciary mark that indicates the position of the DPPH crystal in the sample tube. Center the DPPH sample vertically in the cavity. The center of the black irradiation grid cover corresponds approximately to the vertical center of the cavity.

2. **Open the Interactive Spectrometer Control dialog box.** Click the Interactive Spectrometer Control button in the tool bar and the dialog box will appear. (See Figure 8-5.) We can now optimize some of the parameters and adjustments for the calibration routine.

It is not possible to change the actual Sweep Width while the Set Up Scan is enabled. Change the Sweep Width before the Set Up Scan is enabled.

3. **Set some parameters.** Set the Microwave Attenuator to approximately 25 dB. The Time Constant needs to be set to a low value (less than about 0.16 ms). A Modulation Amplitude of 1 Gauss is usually sufficient. Set the Sweep Width to 100 Gauss. A Receiver Gain of approximately 1 x 10^3 works well.

4. **Click the Enable button for the Set Up Scan.** When this option is enabled, the magnetic field is swept rapidly (up to 50 Gauss) to provide a "real time" display of the EPR spectrum on the screen.

5. **Center your DPPH spectrum in the display.** Adjust the Field slider bar until the signal appears centered in the Setup Scan window. For a microwave frequency of about 9.78 GHz, DPPH resonates at 3480 Gauss. Adjust the Receiver Gain so that the signal fills approximately half of the vertical display range. Make sure that the signal channel is set to 100 kHz modulation and first harmonic detection.

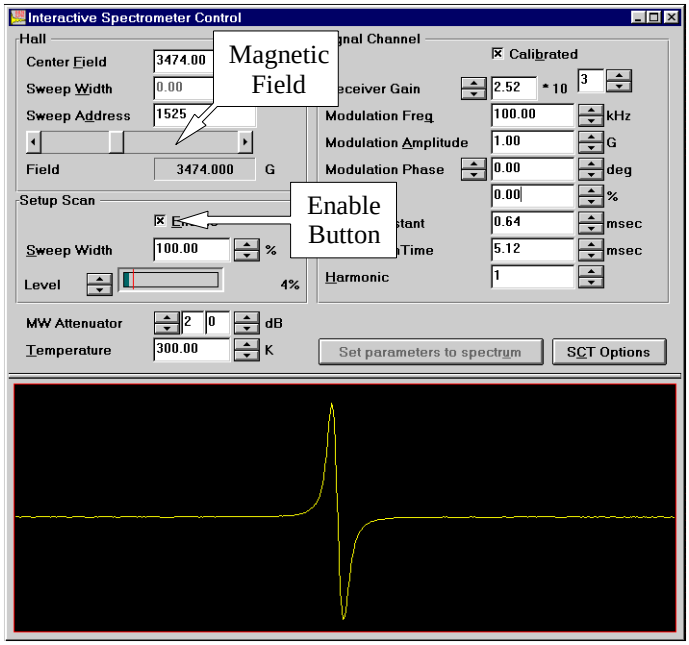


Figure 8-5 The Interactive Spectrometer Control dialog box.

6. **Optimize the DPPH sample position.** Move the sample tube up and down until the maximum signal intensity is attained. (See Figure 8-4.) Avoid moving the sample from side to side. Perhaps the best technique is to loosen the collet nuts for the pedestal and sample tube and move the sample too low. Then use the pedestal to slowly push the sample up. Sometimes the process of moving the sample tube in the cavity can cause the AFC to lose lock. Retune the frequency if this happens. If the signal is clipped, decrease the Receiver Gain. When you have centered the DPPH sample, secure the sample tube by tightening the collet nuts.

If there is no spectrum available, click on the New Experiment button in the tool bar to create a new spectrum. (See Figure 8-6.)

7. **Transfer the parameters.** To set the parameter values to a spectrum, click on the Set parameters to spectrum button. The cursor will turn into the letter P (for Parameter). Place the cursor on a spectrum window and click the left mouse button to copy the parameters to that spectrum. Click the Interactive Spectrometer Control button in the tool bar to close the dialog box.

Figure 8-6 The New Experiment button.

8. **Check the AFC Trap and High Pass Filters.** Click on the Signal Channel Options command in the Parameter drop-down menu. The Signal Channel Options dialog box will appear.

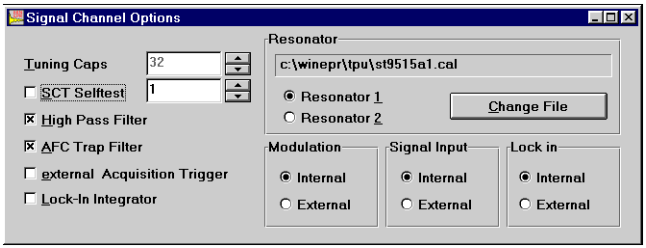


Figure 8-7 The Signal Channel Options dialog box.

The AFC trap filter blocks any frequency signal components at the AFC modulation frequency that may contribute to noise in the EPR signal. The high pass filter suppresses low frequency signal components that may

also contribute to added noise in the EPR signal. These two filters influence the calibration values of the signal channel. By default they are both selected. Ensure that both options are checked. Only under very rare circumstances would you acquire spectra without these filters.

You do not need to edit the Static Field parameter for a signal channel calibration.

9. **Adjust some parameters.** After centering the DPPH sample, most of the parameters should be fairly close to what is needed for the calibration routine. Check the values in the Standard Acquisition Parameter dialog box and modify them so that they correspond to the values in Figure 8-8. The Center Field value may be somewhat different from what is displayed in Figure 8-8, but the Sweep Width must be 100 Gauss.

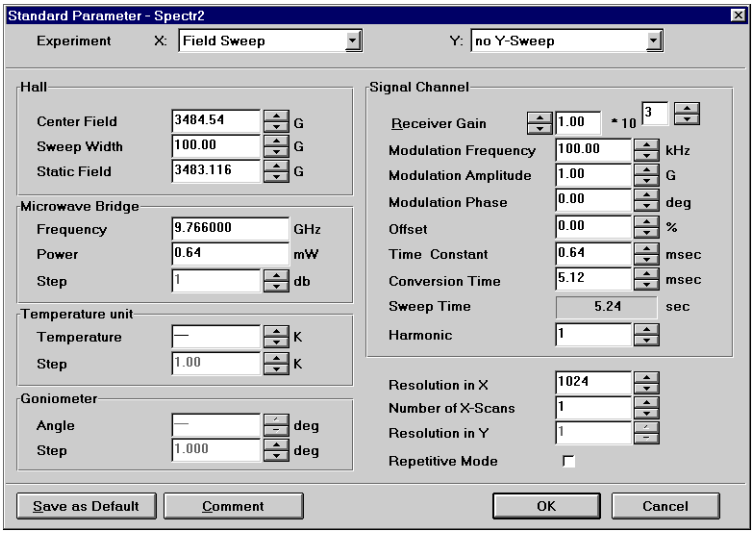


Figure 8-8 Parameters for a signal channel calibration.

10. **Acquire a spectrum.** Click the RUN button in the tool bar.

11. **Adjust the Receiver Gain.** Monitor the Receiver Level while the scan is running. (See Figure 8-9.) If the needle deflects more than 1/4 of the display, lower the Receiver Gain. Reacquire the spectrum and lower the Receiver Gain until the needle does not deflect more than 1/4 of the display. You may have to repeat this last step a few times.

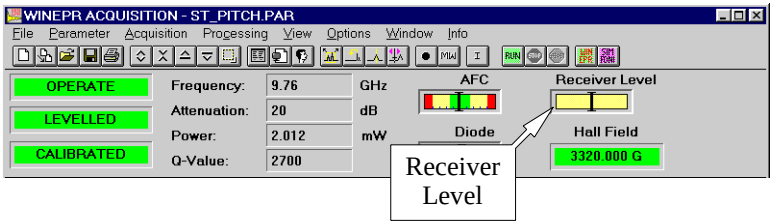


Figure 8-9 The Receiver Level display.

12. **Set the center field.** To interactively set the center field, click the Interactive Change of Center Field Parameter button in the tool bar.(See Figure 8-10.)

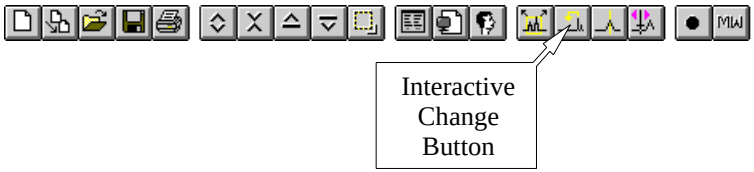


Figure 8-10 The Interactive Change of Center Field Parameter button in the Tool Bar.

Clicking this button creates a marker (vertical line) in the spectrum window that moves with the cursor. Place the cursor where you would like the center field to be and click with the right mouse button. (See Figure 4-13.) This

action replaces the center field value with the magnetic field position of the marker. For further details on this operation consult Section 4.3.2 of this manual.

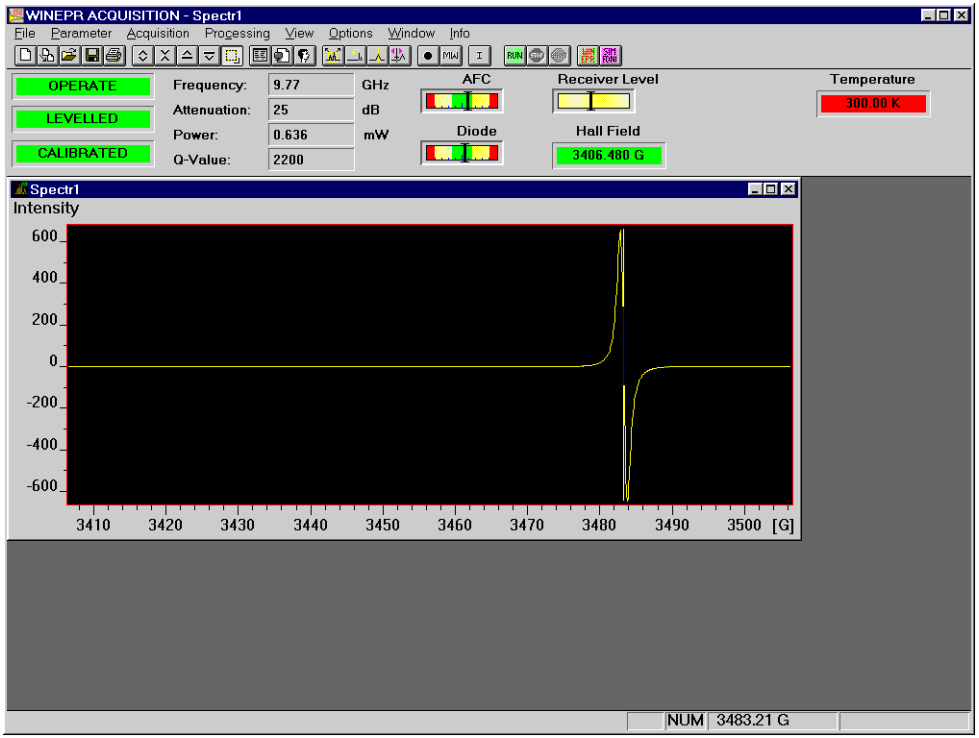


Figure 8-11 The center field marker.

Acquire the spectrum once more. The DPPH signal should now be nicely centered in the spectrum.

Calibrating the Signal Channel 8.2.3

1. **Open the Calibrate Signal Channel dialog box.** Click the Calibrate Signal Channel command in the Acquisition drop-down menu. A new dialog box will appear.

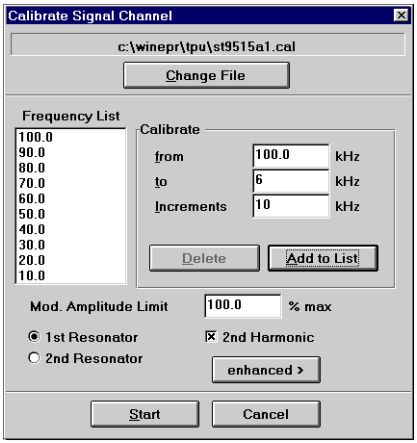


Figure 8-12 The Calibrate Signal Channel dialog box.

2. **Enter the filename for the calibration file.** The calibration file name usually consists of two or three letters that identify the type of cavity (ST for ER 4102ST or TM for ER 4103TM) followed by the serial number of the cavity. This number is found on the back or front of the cavity. Click on the Change File button to open the Open Calibration File dialog box. Signal channel calibration files are normally stored in the tpu subdirectory along with field controller and other calibration files. Enter a filename and click OK.

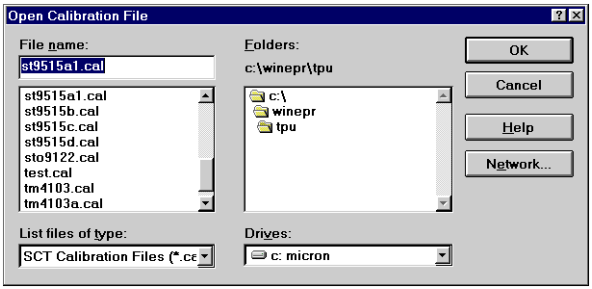


Figure 8-13 The Calibrate Signal Channel dialog box.

3. **Set the frequency limits.** A calibration is required for each modulation frequency that you intend to use. The standard signal channel, the EMX 027 SCT-H, has a range of 100 kHz to 6 kHz in 0.1 kHz steps. Most people will normally run all their spectra using 100 kHz modulation, but under some special circumstances, other frequencies may be desirable. A good approach to take is to calibrate the signal channel every 10 kHz from 100 kHz to 10 kHz. A sufficiently large range of frequencies is then covered for most EPR experiments.

Better safe than sorry. It is a good idea to calibrate the phase of the second harmonic when running the calibration routine.

4. **Choose the harmonics.** The signal channel can produce either a first harmonic (first derivative) or a second harmonic (second derivative) spectrum. If you have no need for second harmonic spectra and wish to save a bit of time in the calibration routine, you may deselect the option to calibrate the phase for the second harmonic by clicking the 2nd Harmonic box. A cross in the box indicates that the option is selected. However, the time-savings are minimal and you never know when you may need a second harmonic display: it is probably best to always calibrate the second harmonic phase.

The ER 4105DR dual cavity is different from the ER 4116DM dual mode cavity. The ER 4116DM has only one set of modulation coils.

5. **Select the resonator.** In almost all cases, the 1st Resonator should be selected. The ER 4105DR dual cavity has two sets of modulation coils. By selecting 1st Resonator or 2nd Resonator, you are selecting the set of modulation coils that are to be calibrated.

6. **Start the calibration routine.** Click the Start button. A new dialog box will appear. (See Figure 8-14.) The spectrometer will then automatically calibrate the signal channel at each of the specified modulation frequencies.

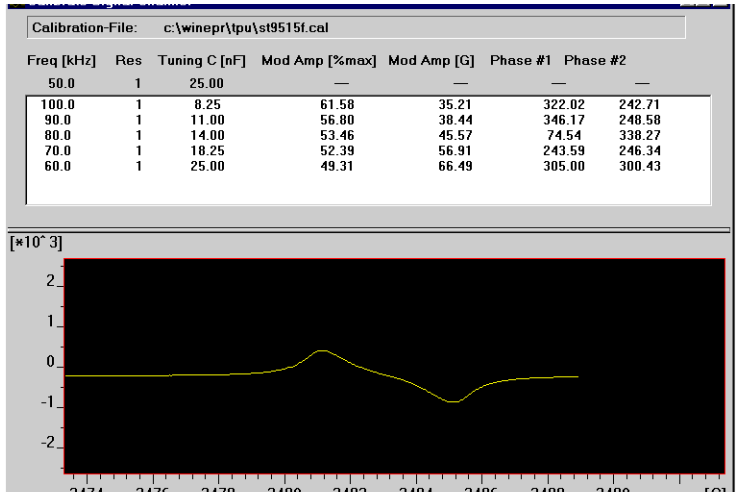


Figure 8-14 The Calibration routine

The calibration file consists of a table of parameter values and settings for each modulation frequency. The first parameter is the modulation frequency. The second column indicates the resonator that was selected in Step 5. For modulation frequencies greater than or equal to 50 kHz, the optimal tuning capacitor value is listed in the third column. The fourth column contains the value of Mod Amp [% max]. Mod Amp [G] in the fifth column is

the measured maximum modulation amplitude when the corresponding Mod Amp [% max] is used. Phase #1 and Phase #2, in columns six and seven respectively, are the phases at which the first and second harmonic signals are nulled.

7. **Check Mod. Amp [G] at 100 kHz.** The calibration routine performs its task sequentially, starting with the highest modulation frequency and continuing for each selected modulation frequency. As each parameter is determined, it is displayed in the table. (See Figure 8-14.) Wait until the 100 kHz calibration is completed and note the value in the fifth column of the table, Mod Amp [G]. This value can allow excessive current to flow through the modulation coils of the cavity at the maximum modulation amplitude, resulting in damaged modulation coils. Compare the Mod Amp [G] at 100 kHz with the values listed for your cavity in Table 8-1. If the value obtained by the calibration routine exceeds the values listed in Table 8-1, first record the values for Mod Amp [G] and Mod Amp [%max] because you will need them for the next step. Then stop the calibration routine by clicking the STOP button in the Tool Bar twice. If the value is less than or equal to the value listed in Table 8-1, allow the calibration routine to continue its task and proceed to Step 9.

Cavity	Maximum Mod. / Gauss at 100 kHz
ER 4102ST	32
ER 4105DR	32
ER 4104OR	32
ER 4116DM	10
ER 4103TM	16
ER 4108TMH	16
ER 4106ZRC	10
ER 4106ZRAC	10
ER 4107WZC	10
ER 4107WZAC	10
ER 4115ODC	10
ER 4115ODAC	10
ER 4122SHQ	15
ER 4114HT	10
ER 4117D-MVT	10
ER 4117D-R	10
ER 4109EF	10

Table 8-1 Maximum modulation amplitude for EPR cavities.

8. **Set the Mod. Amplitude Limit.** The Mod. Amplitude Limit parameter in the Calibrate Signal Channel dialog box allows you to limit the size of the signal sent to the modulation amplifier to prevent any danger of burning out the coils. To calculate a safer value for Mod. Amplitude Limit use the following formula:

$$\text{Mod Amplitude Limit} = \text{Mod Amp[\%max]} \frac{\text{Max Mod}}{\text{Actual Mod}}$$

where Mod Amp[%max] is the value determined by the calibration routine, Actual Mod is the value for Mod. Amp [G] determined by the calibration routine, and Max Mod is the maximum modulation amplitude listed for your cavity in Table 8-1. Return to Step 1. (*e.g.* start the calibration routine again) and enter this new value for Mod. Amplitude Limit. Continue from Step 2. through Step 7. as before.

9. **Finish the Calibration.** When the routine is finished the message Acquisition Done! will appear in the info line. Double click the control menu box in the upper left hand corner of the window to close the window. The signal channel is now calibrated for your cavity and the data saved in the calibration file. The next time that you start the WIN-EPR Acquisition software, this calibration file will be the default calibration file.

System Performance Tests 9

This chapter describes procedures for testing the performance of your Bruker EPR spectrometer. The first test measures the spectrometer's sensitivity. The procedure is especially designed to test as many of the components of the spectrometer as possible with one simple test. It therefore gives you a good indication of the overall health of your spectrometer. It is also an excellent criterion for comparing the sensitivity of different spectrometers. The second test measures the background signal of the cavity. Should your spectrometer or cavity not meet specifications, first consult Chapter 7 on troubleshooting. If none of the hints solve the problem, contact your local Bruker EPR service representative.

Signal to Noise Ratio Test 9.1

The signal to noise ratio test is an important part in maintaining your spectrometer. It is also helpful in diagnosing possible problems you may encounter especially when you deal with very weak signals or quantify your EPR signals. A standard signal to noise ratio test uses the ER 4102ST standard cavity and the weak pitch sample that was shipped with your spectrometer. The test measures the EPR signal intensity (peak-to-peak height) of the weak pitch sample at low microwave power (12 db) and then measures the noise level under the same conditions except higher microwave power (0 db) and higher receiver gain to characterize the noise better. The formula for calculation of signal to noise ratio is:

$$\frac{S}{N} = \frac{A_S}{A_N} \times \frac{G_N}{G_S} \times \sqrt{\frac{P_N}{P_S}} \times \frac{2.5}{\sqrt{T} \times C}, \qquad [9\text{-}1]$$

where A_S and A_N are the peak to peak height of the weak pitch and amplitude of the noise respectively. G_S and G_N are the receiver gains used in signal and noise measurements respectively. We use their ratio to correct for the gain difference. P_S and P_N are the powers used in two measurements and we use the square root of the ratio of powers to correct for the power difference. The factor of 2.5 translates the peak-to-peak noise level to a RMS (Root Mean Square) noise level. T is the time constant (in seconds) and we use the square root of the time constant to normalize the S/N to a one second time constant. C is the weak pitch correction factor that is printed on the label of the weak pitch sample. The standard instrument settings for signal and noise measurements are listed in Table 9-1. There is a built-in subroutine to measure the signal to noise ratio which has the default values of standard settings. If you want to measure the amplitudes of the signal and noise on a print out by hand, make sure that you use the same scale for both signal and noise spec-

tra. Otherwise you need to multiply the result by the ratio of the scales.

Parameter	Signal Measurement	Noise Measurement
Modulation Amplitude	8.0 G	8.0 G
Modulation Frequency	100 kHz	100 kHz
Receiver Gain	2.0×10^5	5.0×10^5
Phase	0	0
Time Constant	1310.72 ms	1310.72 ms
Conversion Time	163.84 ms	163.84 ms
Center Field	3480 G	3300 G
X Axis Setting	Field Sweep	Time Scan
Sweep Width	50 G	--
Resolution of X Axis	1024 points	1024 points
Microwave Attenuation	12 dB	0 dB

Table 9-1 Parameters for Signal/Noise Measurements.

Preparing for the S/N Test 9.1.1

1. **Install an ER 4102ST standard cavity.** (See Section 5.2 for instructions.) The specification for the signal to noise ratio is based on an ER 4102ST standard cavity and using the weak pitch sample. We strongly suggest using the standard cavity for this standard test and keep a record and verify the specification periodically. If you use other types of cavities to do the signal to noise ratio test the results and the settings will be different due to different Q values and the microwave field distributions of the cavities.

The calibration factor is found on the weak pitch sample's label. It is listed as:

$C = C_0 \times$ calibration factor

It is usually approximately equal to one and corrects for variations in the sample concentration.

2. **Insert the weak pitch sample**. Copy down the calibration factor posted on the label of the weak pitch before you insert it. The weak pitch sample should be inserted in the cavity until the bottom of the label and tape on the sample tube is flush with the collet. You also should use the pedestal to hold the weak pitch rigidly.

3. **Turn on the instrument and tune.** Turn on the instrument if it is not on yet. Tune the microwave bridge and the cavity. It is best to wait several hours, because the spectrometer is most sensitive and stable after it has achieved thermal equilibrium.

4. **Set the AFC depth**. You can find the AFC depth adjustment knob on the back of the microwave bridge. (See Figure 9-1.) Full scale is ten. Set the AFC depth (or amplitude) at around 2.

5. **Check the signal channel options settings.** Open the Signal Channel Options dialog box. Make sure you have the right calibration file for the standard cavity you are using. High Pass Filter and AFC Trap Filter (See Figure 9-2.) are checked in the default settings. In case the default settings have been changed, set them back.

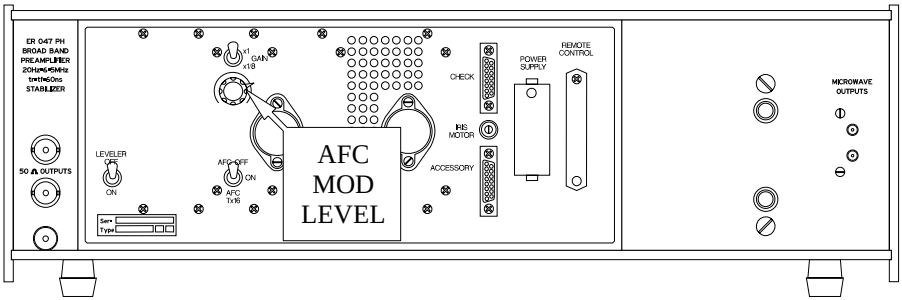


Figure 9-1 Location of the AFC MOD LEVEL potentiometer

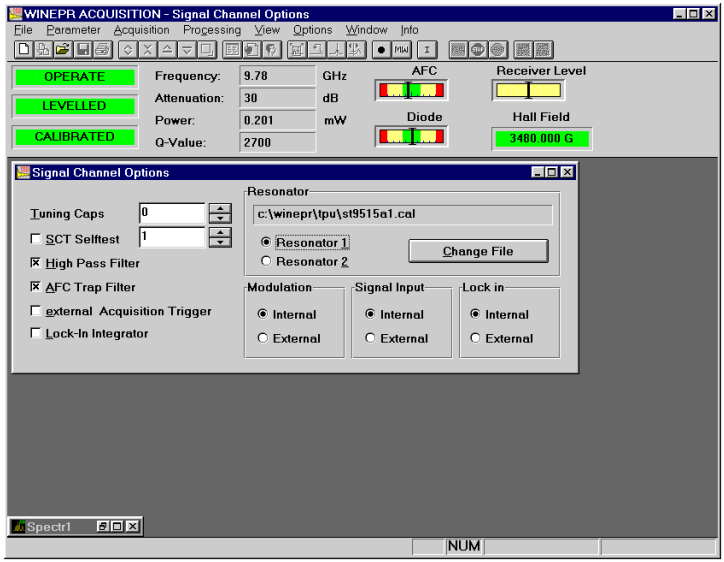


Figure 9-2 Signal Channel Options.

Measuring the Signal to Noise Ratio 9.1.2

1. **Open the Signal/Noise Ratio Test window.** Open the Signal/Noise Ratio Test window under the Acquisition drop-down menu. (See Figure 9-3.) The window has two empty spectra and each one contains a set of default parameters for signal or noise measurement. Click either one of the windows with the left mouse button to activate that window. The parameters shown on the right will be assigned to that measurement.

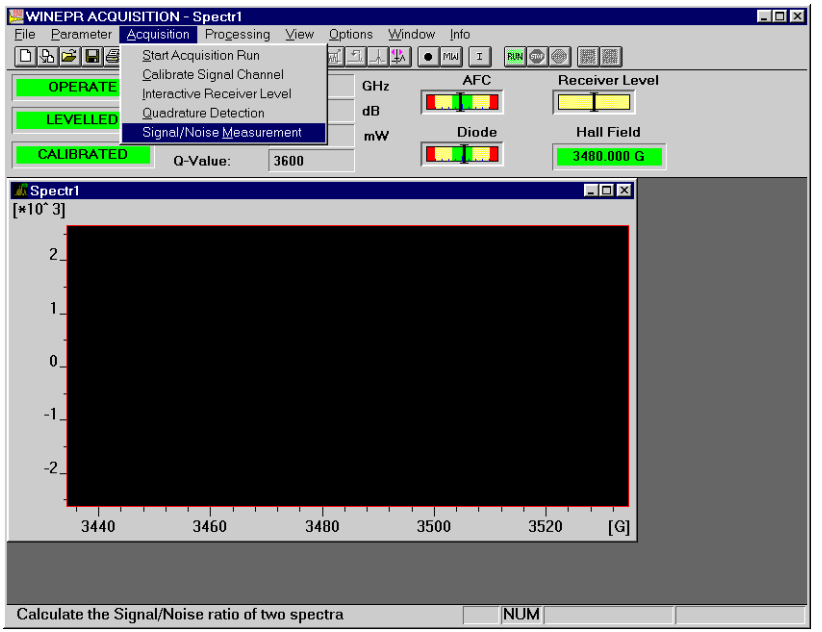


Figure 9-3 Open Signal/Noise Measurement window.

2. **Input the calibration factor for the weak pitch sample**. Enter the calibration factor you copied in

Step 2. of the previous section into the Weak Pitch factor box. (See Figure 9-4.)

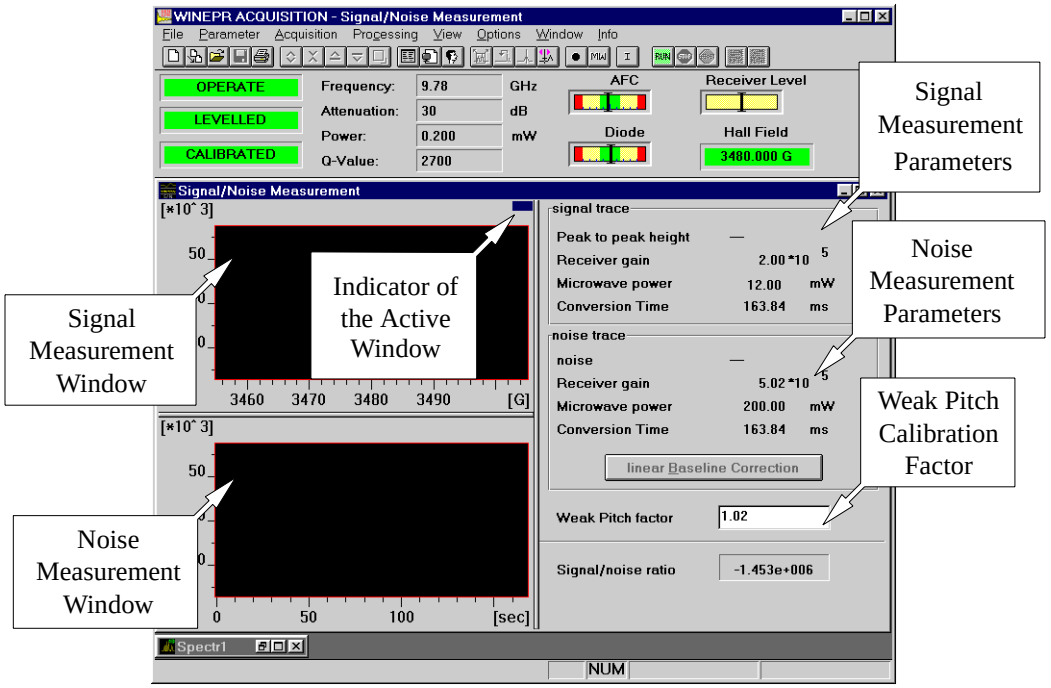


Figure 9-4 Signal/Noise Measurement Window.

3. **Activate the signal measurement.** Click the signal window (the upper one). A blue bar will appear on the right upper corner. Check the parameter settings by opening the Standard Parameter dialog box. The parameters should look like those in Figure 9-5.

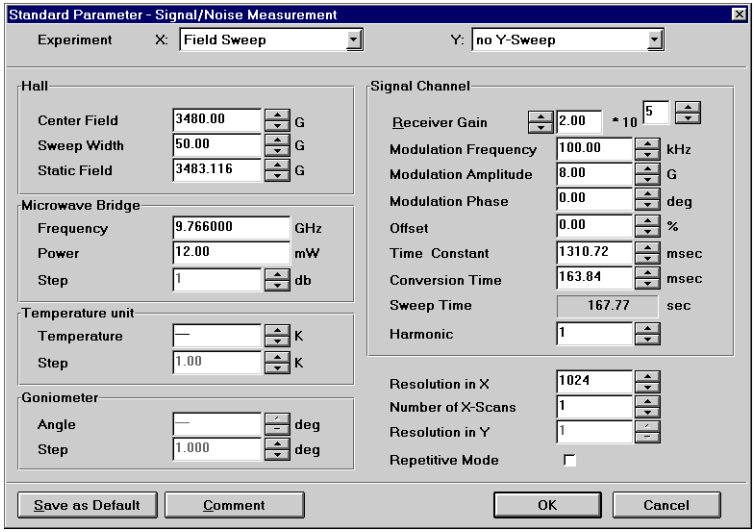


Figure 9-5 Parameters for signal measurement.

4. **Set a time delay.** Since a very long time constant is used, set a delay time of 2-5 seconds to avoid overshoots or undershoots in the first few data points when you acquire the spectrum. Open the Experimental Options dialog box (found in the Parameter drop-down menu) and set the Delay before each sweep option and a delay of two to five seconds. (See Figure 9-6.) We also advise you to select MW Fine Tune before each sweep option to ensure the acquisition is made under proper coupling conditions.

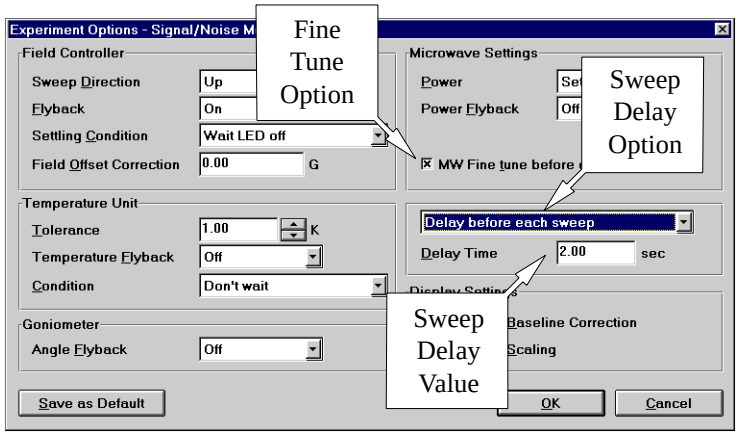


Figure 9-6 Set Experimental Options.

See Section 4.3.2 for help in setting center fields and Section 4.5 for help with interactive spectrometer control.

5. **Acquire a signal spectrum.** Click the RUN button in the tool bar to acquire a weak pitch spectrum. (See Figure 9-7.) If the spectrum is off center you can use the center field tool to set the correct field center. If there is a large offset you can open the Interactive Spectrometer Control dialog box to adjust the offset to the proper position where the indicator of the Receiver Level is in the middle. Do not forget to click the Set Parameters to the Spectrum button and move the pointer to the signal measurement window and click the left mouse button again.

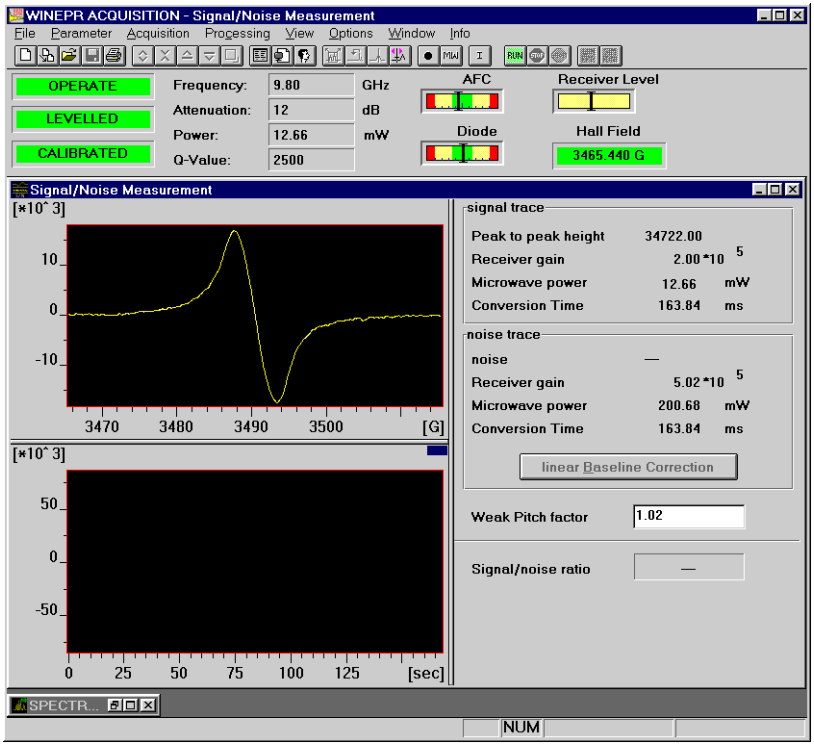


Figure 9-7 Signal measurement.

6. **Activate the noise measurement.** Click the lower window to activate the noise measurement window.

7. **Check the parameters.** Open the parameter dialog box. Make sure the X axis is set to Time Scan, the power is 200 mW, gain is 5 x 10^5 and the field center at 3300 G. The other parameters should be similar to that in signal measurement. (See Figure 9-8.)

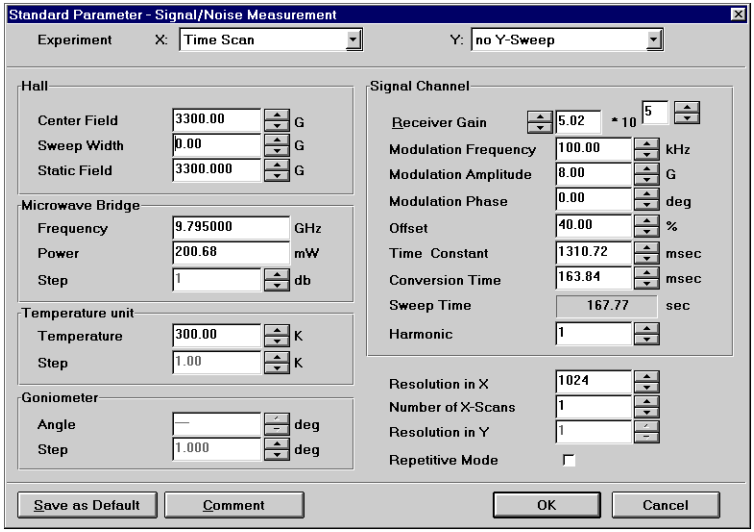


Figure 9-8 Parameters for noise measurement.

See Section 4.5 for help with interactive spectrometer control.

8. **Get ready to acquire a noise spectrum.** Click the RUN button in the tool bar to acquire the noise spectrum. Frequently the baseline will drift since 200 mW microwave power is going to heat up the cavity and the sample. Wait a few minutes to achieve thermal equilibrium. Check the tuning and coupling of the system. Retune the system if necessary. You may also have a rather large offset due to the excessive power and high gain. Use the interactive box to make the offset adjustment so that the indicator of the receiver level is in the middle. Click the left mouse button on Set Parameters to Spectrum, move the pointer to the noise measurement window, and click again. If you experience overshoots or undershoots, you can set a 2-5 second delay time in the Experimental Options box as in Step 4.

9. **Acquire a noise spectrum.** Click the RUN button in the tool bar and acquire the noise spectrum again. Two horizontal lines will automatically emerge indicating the noise level. If the baseline still drifts you can click the linear baseline correction button to compensate for linear drifts.

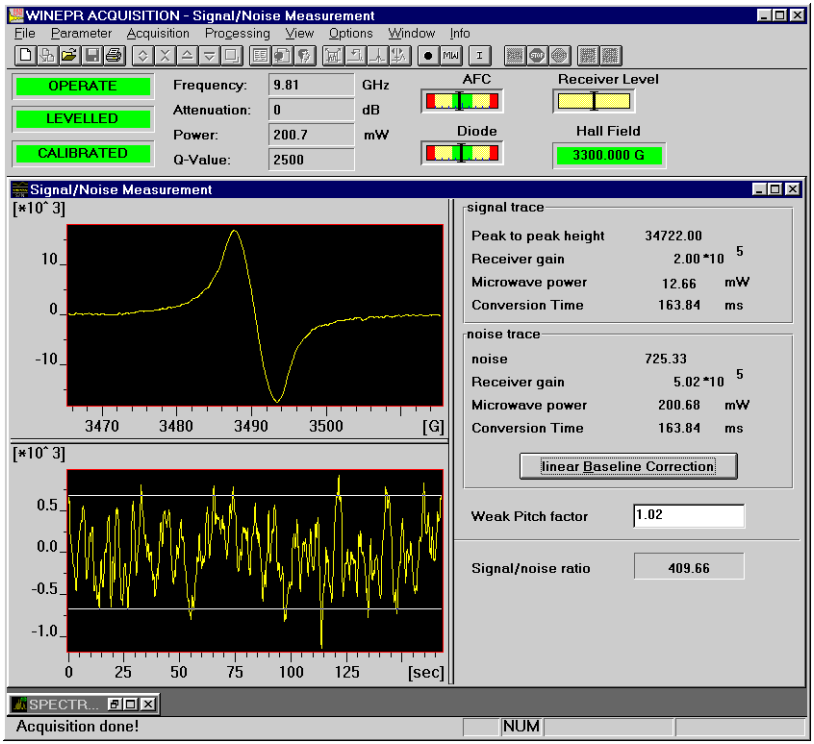


Figure 9-9 Noise measurement and the final result.

10. **Check the S/N ratio**. On the right panel the results of the signal intensity and noise level measurements will automatically appear. At the bottom of the panel, the automatically calculated signal to noise ratio will be displayed in the box. (See Figure 9-9.) The signal to noise ratio should be higher than 330 to meet the specifications of the Bruker EPR instrument. If the result is lower than this value, consult Chapter 6 and Chapter 7. Sometimes a large cavity background signal can significantly decrease the test result. Refer to the next section (Section 9.2) and run a cavity background signal test to verify this. If those hints do not help, contact your local Bruker service representatives.

Cavity Background Signal Test 9.2

Cavity background signals can sometimes be disturbing, particularly when they overlap with your EPR signals or with the area you need to integrate. They can distort the EPR signals of your sample and make quantification difficult. The best way to avoid these problems is to keep your cavity clean. Here we provide a standard procedure to test your cavity background signal. The standard cavity background signal test compares the weak pitch signal with the spectrum acquired with an empty cavity over a wide scan range. The parameter setting for a standard test is shown in Table 9-2. The ratio of the cavity background signal over the peak-to-peak height of the weak pitch signal should be less than 1/4 to meet the specifications.

Preparing for the Background Signal Test 9.2.1

1. **Install an ER 4102ST standard cavity.** (See Section 5.2 for instructions.) The specification for the background signal is based on an ER 4102ST standard cavity and using the weak pitch sample. We strongly suggest that you keep a record and verify the specification periodically.

2. **Insert the weak pitch sample**. The weak pitch sample should be inserted in the cavity until the bottom of the label and tape on the sample tube is flush with the collet. You also should use the pedestal to hold the weak pitch rigidly.

3. **Turn on the instrument and tune.** Turn on the instrument if it is not on yet. Tune the microwave bridge and the cavity. It is best to wait several hours, because the spectrometer is most sensitive and stable after it has achieved thermal equilibrium.

4. **Create a new spectrum window, if needed.** If there is no empty spectrum window, create one by clicking on the Create New Spectrum button in the tool bar.

Performing the Background Signal Test 9.2.2

1. **Open the parameter option dialog box.** Set the parameters for Weak Pitch Measurement as indicated in Table 9-2. (See Figure 9-10.)

Parameter	Weak Pitch Measurement	Background Measurement
Modulation Amplitude	8.0 G	8.0 G
Modulation Frequency	100 kHz	100 kHz
Receiver Gain	adjust	adjust
Phase	0	0
Time Constant	1310.72 ms	1310.72 ms
Conversion Time	163.84 ms	163.84 ms
Center Field	3480 G	2600 G
Sweep Width	50 G	5000 G
Resolution of Field Axis	1024 points	1024 points
Microwave Attenuation	3 dB	3 dB

Table 9-2 Parameters for Background Signal Measurement.

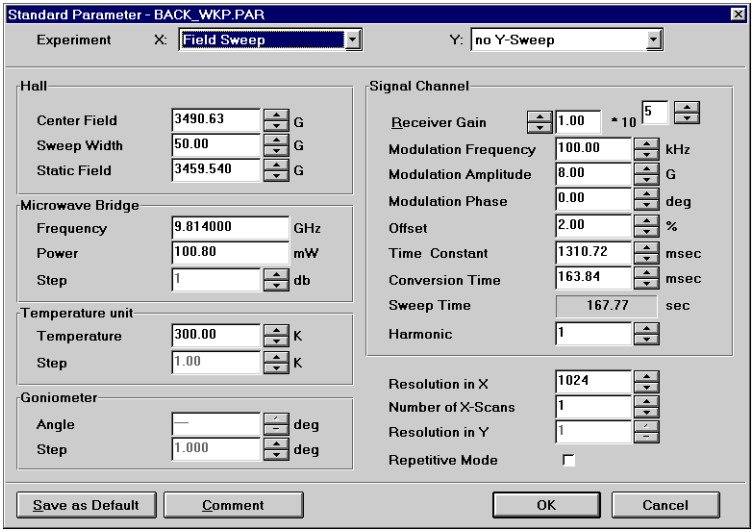


Figure 9-10 Set the parameters for weak pitch sample.

2. **Set receiver gain properly.** Since the microwave power (3 db) is higher than in the signal/noise ratio test, you may need to adjust the receiver gain accordingly. The suggested receiver gain is 1×10^5.

3. **Set a time delay.** Since a very long time constant is used, set a delay time of 2-5 seconds to avoid overshoots or undershoots in the first few data points when you acquire the spectrum. Open the Experimental Options dialog box (found in the Parameter drop-down menu) and set the Delay before each sweep option and a delay of two to five seconds. (See Figure 9-11.)

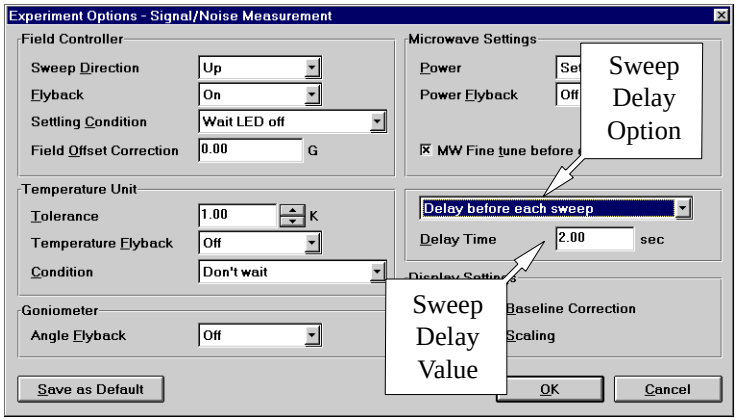


Figure 9-11 Set Experimental Options.

4. **Acquire a weak pitch spectrum.** Click the RUN button in the tool bar to acquire a weak pitch spectrum. (See Figure 9-12.)

5. **Adjust Receiver Gain, if needed.** If the weak pitch signal clipped, return back to Step 2. and reacquire the spectrum.

6. **Adjust the offset, if needed.** If there is a large offset you can open the Interactive Spectrometer Control dialog box to adjust the offset to the proper position where the indicator of the Receiver Level is in the middle. Do not forget to click the Set Parameters to the Spectrum button and move the pointer to the signal measurement window and click the left mouse button again. Reacquire the spectrum

See Section 4.5 for help with interactive spectrometer control.

7. **Save the spectrum.** Save the spectrum on the hard disk for future reference.

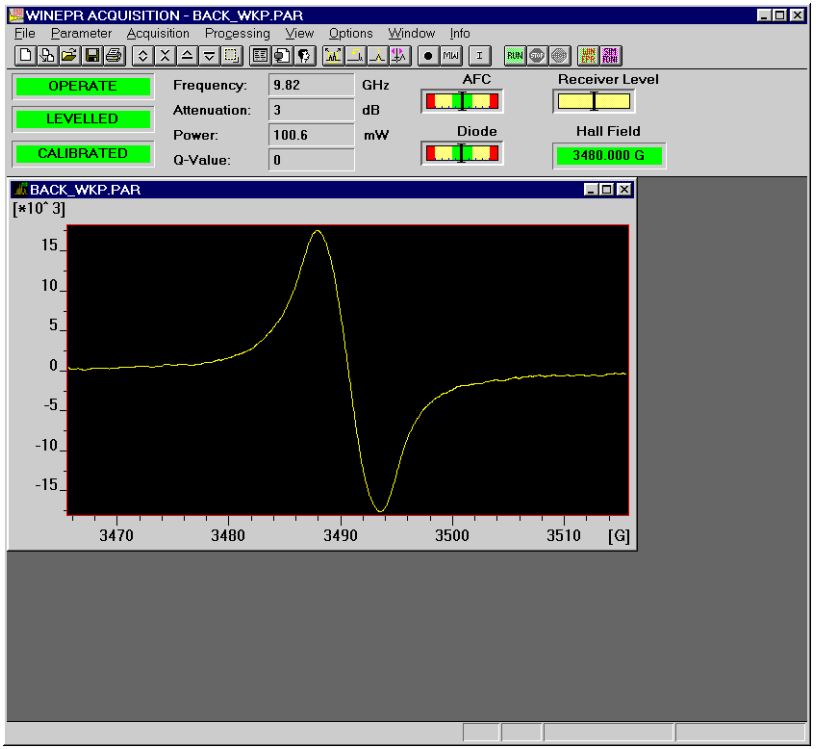


Figure 9-12 Acquire a weak pitch signal.

8. **Open Microwave Control dialog box and set to Stand by.**

9. **Remove the weak pitch sample and retune the bridge and cavity.**

10. **Duplicate the weak pitch spectrum window**. Click the Duplicate button in the tool bar.

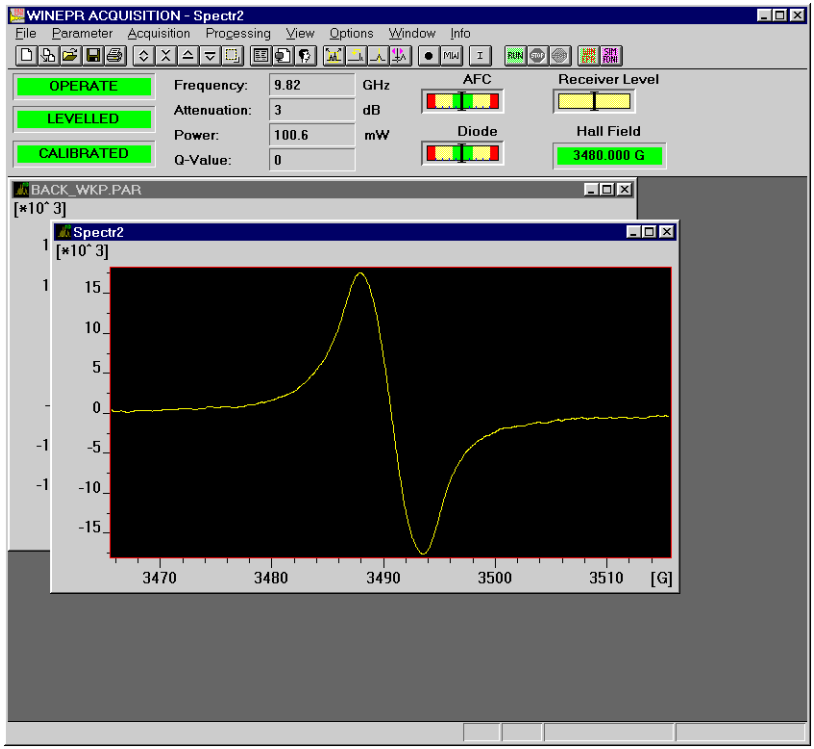


Figure 9-13 Duplicate weak pitch spectrum.

11. **Change the Center Field and Sweep Width.** Open the Experiment parameter dialog box. Change the Center Field to 2600 G and the Sweep Width to 5000 G.

Other parameters should be the same as that for the weak pitch measurement. (See Figure 9-14 and Table 9-2.)

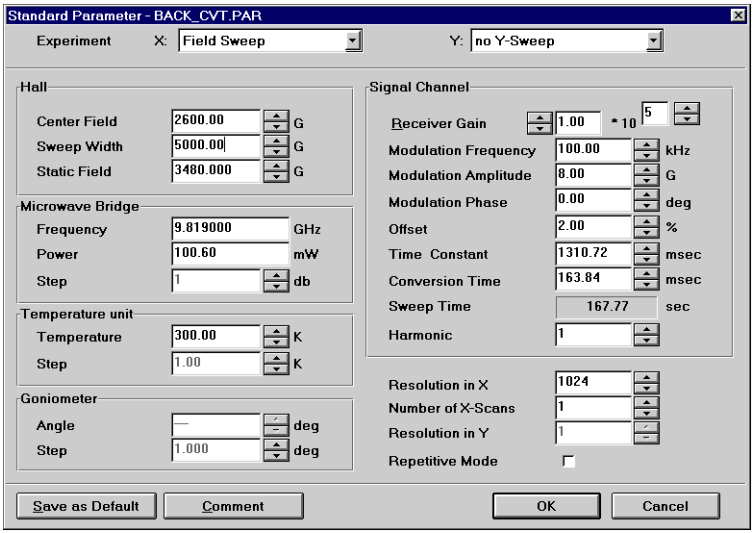


Figure 9-14 Parameters for cavity background signal measurement.

12. **Set a time delay.** Set a 2-5 seconds time delay in the Experiment Options box as in Section 9.2.2., Step 3.

13. **Acquire a cavity background spectrum.** Click the RUN button in the tool bar to acquire the cavity background signal. (See Figure 9-15.)

14. **Save the spectrum.** Save the spectrum on the hard disk for future reference.

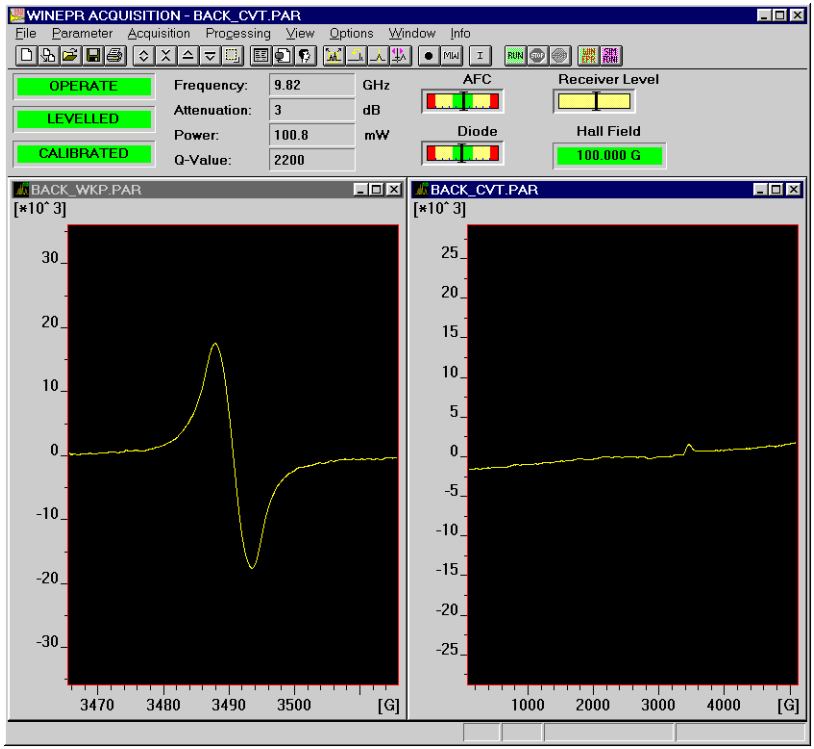


Figure 9-15 Acquire the cavity background signal.

15. **Transfer the spectra to WinEPR.** Transfer the two spectra you acquired to WinEPR. You can either open the WinEPR program and then load the data files you just saved or you can click the Transfer to WinEPR button in the tool bar which will automatically launch the WinEPR program and transfer the spectrum of the active window. To transfer the other spectrum you need to activate that spectrum window in Acquisition program by clicking the

spectrum window and then click the Transfer to WinEPR button in the tool bar again. The WinEPR application will appear. (See Figure 9-16.)

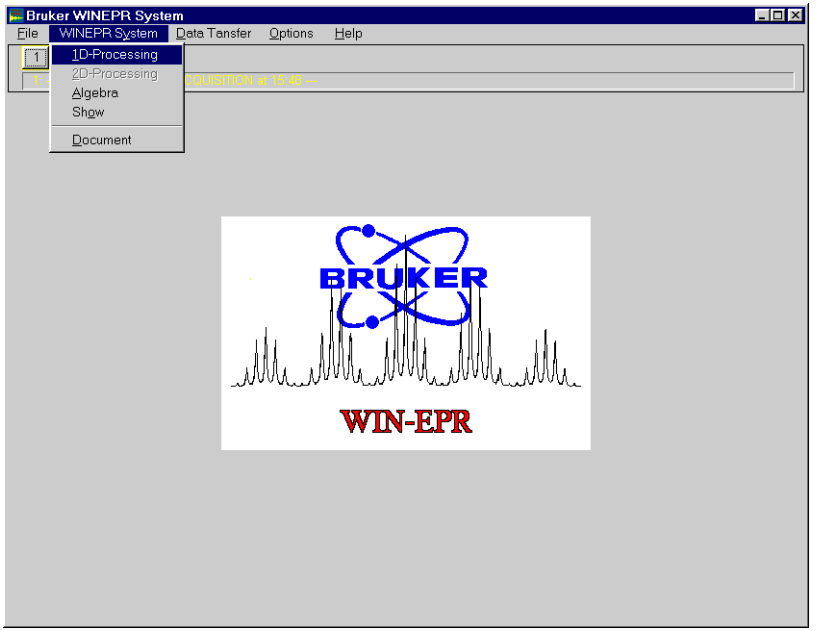


Figure 9-16 Transfer to the WinEPR for data processing.

16. **Click 1D processing under WINEPR System.** Select the cavity background spectrum.

17. **Measure the cavity background signal.** Click Expand under Display. A box contains Expand Display Values will appear. On the right side of the box there are low val and high val of the Y-Scale. The difference between these two values is the signal height of the cavity background signal. (See Figure 9-17.)

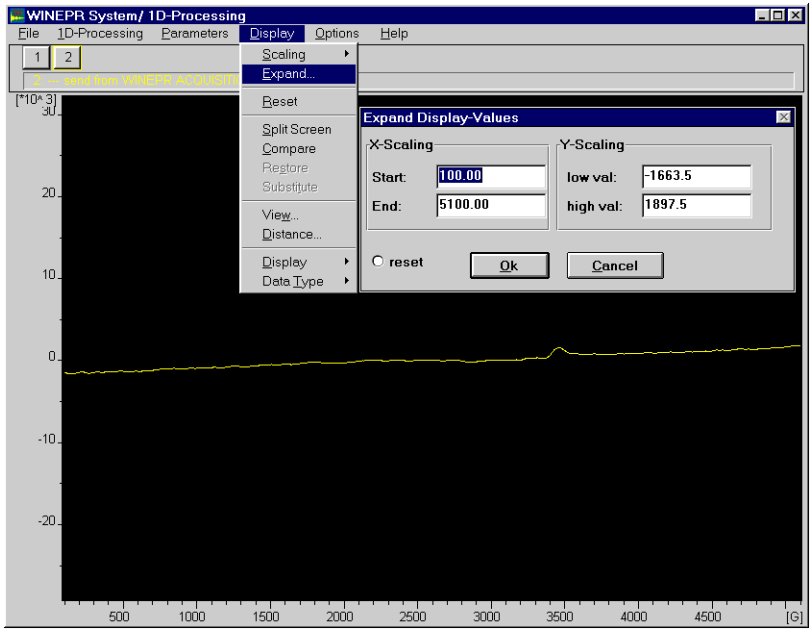


Figure 9-17 Measure the signal height of the cavity background signal.

18. **Measure the weak pitch signal.** Click the weak pitch spectrum and repeat the same procedure to get the signal height of the weak pitch signal.

19. **Calculate the result.** The ratio of the cavity background signal over the weak pitch signal is the test result.

$$\frac{\text{cavity background signal (high val - low val)}}{\text{weak pitch signal (high val - low val)}} < \frac{1}{4}$$

The ratio must be less than 1/4 to meet the specifications. If the ratio is greater than 1/4 contact your local Bruker EPR service representative.

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